comparison to potential risks from MMT exposure; that is, a net risk assessment was not performed.

The major issue of concern to EPA, expressed in the ORD report, is with exposure of the most highly exposed portions of the population to manganese in the form of Mn_3O_4. In addition to preparing the November 1 report, ORD organized a workshop to explore the status of knowledge regarding manganese and MMT and to prioritize research needs. This paper was prepared for and presented at the workshop.

Purpose of this Report

This report analyzes the degree to which use of MMT in unleaded gasoline would increase or decrease exposures to hazardous auto emissions. This analysis is conducted for the population as a whole and for highly exposed populations, based on currently available information. The objective is to compare potential risks from the use of unleaded gasoline containing MMT with risks from the use of unleaded gasoline without MMT.

Summary of Findings

When exposures to hazardous substances (and their potential risks) associated with unleaded gasoline are considered in comparison to exposures and risks with MMT use,

the analysis clearly supports the view that MMT use is beneficial from an environmental, health, and economic basis. This is true for average individuals and for those in high exposure groups. Existing data and analyses indicate that MMT use would provide a positive net benefit to society as a whole and to those who experience peak exposures to automobile emissions.

New Risk Versus Net Risk

The ORD report considers potential risks from MMT use, but from a narrow perspective. The question that the ORD document seeks to answer is whether it is "possible to state definitively whether a significant health risk from inhalation exposure to manganese will, (or will not) occur with usage of MMT." Notwithstanding the difficulty in proving that risks do not exist, the effect of MMT use on risk, including those risks that would decrease as well as those that would increase, is a more relevant public policy question.

While potential health and environmental benefits from MMT use (such as reduction in the benzene content of gasoline) were acknowledged in the ORD analysis, no analysis was made comparing potential risks from exposure to manganese emissions with the risk reductions associated with reduced emissions of other pollutants. Although ORD did not attempt to assess net risks in their analysis of MMT use, EPA endorses a systems viewpoint for examining the health and environmental aspects of transportation fuels in

its most recent policy position, "ORD Interim Alternative Fuels Research Plan," Draft No. 1, of May 9, 1990.

The question considered here is whether an assessment based on present knowledge indicates that use of MMT adds to, or reduces, environmental and health risk when the system is considered as a whole (i.e., including both auto and refinery emissions). In the course of assessing the net impact from the use or nonuse of MMT, a key step to avoid bias in the analysis is to ensure that equivalent assumptions, in terms of conservatism or non-conservatism, have been applied. For this reason, EPA risk analyses and EPA-developed or approved methods and assumptions for quantitative risk assessment are applied to the extent that data and methods permit.

One difficulty with a systematic assessment of risks from unleaded gasoline with and without MMT is that the effects considered differ in their nature. Current risk assessment techniques do not permit equivalent quantification of risks due to exposures to carcinogens and non-carcinogens. Thus, there is no method available by which exposures to non-carcinogenic substances such as manganese can be compared to risks from carcinogens on a uniform basis. It is even more difficult to evaluate and compare other effects of MMT use such as reductions in oil imports or in CO_2 emissions. No attempt is made here to convert all impacts to a common scale, i.e., dollars. Instead, changes in health or environmental impact are summarized in the units best suited in each case.

Analytic Scope

The analysis includes the following effects:

- **Carcinogenic Auto Emissions** -- EPA has analyzed and expressed concern regarding five hydrocarbons emitted by automobiles: formaldehyde, benzene, acetaldehyde, 1,3-butadiene, and polycyclic organic matter (POM). Emissions data for autos fueled with unleaded gasoline with and without MMT are available for the first four of these substances, but not for POM. Emissions of POM from cars using unleaded gasoline with and without MMT were below detection in tests conducted by Southwest Research Institute for Ethyl, and POM was not included in published assessments of carcinogenic risks from automobiles carried out by EPA staff. This analysis therefore considers cancer risks from the first four hydrocarbons noted.

Plausible upper bound risks are estimated based on EPA unit risk factors and methods. The analysis uses data and results obtained by a study by EPA Ann Arbor staff (Adler and Carey, 1989).

- **Noncarcinogenic Auto Emissions** -- Exposures to CO, NO_x, particulates and manganese are assessed. The results of the analysis are expressed in terms of ratios to EPA standards for each of these substances. For CO, exposures are reported as a ratio of the eight-hour ambient standard; NO_x and particulate exposures are described in

relation to their annual ambient standards; manganese exposure is described in comparison to the manganese inhalation reference concentration (RfC). Although EPA has not published RfCs for the hydrocarbon carcinogens named above, California's Department of Health Services has set inhalation reference concentrations for use in risk assessments for formaldehyde, benzene, and acetaldehyde. These RfCs reflect concern with risks for effects other than cancer.

■ **Other Adverse Effects** -- Although the major concern expressed in the ORD assessment was with exposures to manganese emitted by cars, it was noted that MMT is toxic and that there is the possibility of direct exposure or of MMT reaching ground water. Risk from MMT, after it has been added to gasoline, was not cited as a concern by ORD because there will be no identifiable difference in gasoline risk or toxicity with MMT due to the small quantity used. The potential risks from MMT exposure are not quantified here; qualitatively they appear to be small in comparison to other effects.

■ **Other benefits** -- Because MMT is highly effective in improving octane, there are environmental, energy, and economic benefits in refinery operations with MMT use. The benefits include reductions in refinery emissions of CO, NO_x, SO_x, CO_2 and particulates and a significant reduction in refinery energy input. Energy savings are estimated to be 30 million barrels of oil a year.

Analytic Approach and Assumptions

The analysis is based on the following qualifications and assumptions:

- Throughout this analysis, cancer risk estimates should be interpreted as plausible upper bounds, based on EPA unit risk factors and on risk assessment methods developed by EPA's Carcinogen Assessment Group.

- It is assumed that all U.S. unleaded gasoline uses MMT at 1/32 gram manganese per gallon. This assumption will overestimate MMT use if significant use of reformulated or other unleaded gasoline not containing MMT occurs. It is also assumed that 20% of manganese in MMT is emitted from the tailpipe. The actual emission rate varies with driving cycle; the average manganese emission rate observed by EPA in its testing is around 15%. It is also assumed that the fleet mileage is 20 miles per gallon.

Exposures and risks (and risk reductions) are calculated for unleaded gasoline with and without MMT for two groups: the U.S. population as a whole and a high exposure group. The U.S. population exposure and risk calculations are based on average exposures. The high exposure group calculations assume that some group is exposed to high concentrations of auto emissions, including comparatively high exposures to the four carcinogens listed above, to CO, NO_x and particulates, and to manganese.

For CO and NO_x, emissions reductions associated with MMT use were based on Ethyl's 48 car fleet data through 75,000 miles (data on the fleet tests were provided in the 1990 waiver application). As noted, there is no accepted approach to quantify risks from exposures to non-carcinogens. The standard method to describe such exposures is through use of a Hazard Index (HI), which is the ratio of exposure to the RfC or other applicable standard. For this reason, exposures to CO, NO_x, particulates and manganese are expressed in terms of their ratios to exposure standards for each substance. The details of these calculations are given in following sections.

In this analysis, exposures are assumed to be proportional to the rates of emissions, with corrections added to account for background and non-auto sources. The estimate of the relation of emissions to exposure for gaseous emissions is based on the EPA modified NAAQS Exposure Model (NEM) results reported in Adler & Carey. Because manganese emissions are in the form of particulates while other emissions of interest are gases, this assumption may overestimate manganese exposures. This is because particulates are typically removed from air more rapidly than are gases.

- **Average Exposures**

The average case calculation for unleaded fuel (without MMT) uses emissions rates and average exposures for carcinogens as measured and calculated in Adler and Carey for 1995. Emission rates in Adler and Carey are based on the model MOBILE4; this model

considers improvements in emissions performance and the changing mix of vehicles over time. Emission reductions for the four hydrocarbons (formaldehyde, benzene, acetaldehyde, and 1,3-butadiene) due to MMT use are based on speciation data measured at the Southwest Research Institute.

In this current analysis, average exposures are based on the ratio of automobile emissions to environmental exposure calculated for the four carcinogens by Adler and Carey using NEM. Adler and Carey estimate a range for both emissions and for exposures. The ratio of average concentrations in $\mu g/m^3$ to emissions in grams/mile implicit in Adler and Carey's analysis is calculated here for the range minima, maxima, and arithmetic midpoints. For all three cases, (i.e., minima, midpoint, and maxima) the ratio of concentration in $\mu g/m^3$ to emissions in gm/mile is between 33 and 35 for formaldehyde, acetaldehyde, and 1,3-butadiene. This is generally consistent with, but slightly higher than, the rule of thumb used in the ORD analysis, that emissions in gm/mile multiplied by 30 gives average concentrations in $\mu g/m^3$.

The ratio of concentration to emission for benzene is more variable; for the range minimum, estimates for emissions and concentrations the ratio in Adler and Carey is 35, for the range midpoints the ratio is 45, for the upper end of the range the ratio is 53. The fact that the ratio of concentration to exhaust emissions is higher for benzene than for the other three hydrocarbons is consistent with the fact that there are benzene exposures due to evaporative and refueling emissions as well as to exhaust emissions.

Adler and Carey's range midpoint values for emissions and exposure to the four carcinogens were used for calculations of cancer risk and risk reductions in this paper. Manganese, CO, NO_x and particulate exposures are calculated based on a ratio of 1:35 between emissions and average concentrations, with appropriate adjustments for non-auto sources.

- **High Exposure Groups**

The high exposure group for manganese is also the high exposure group for all other hazardous automobile emissions. Those people who would be exposed to comparatively high levels of manganese are also exposed to high levels of many other pollutants of concern. The point of this observation is not to trivialize the potential risk from manganese by comparing it to other, larger risks. Instead, it is to note that those who are exposed to the highest levels of manganese are also those who would receive the greatest benefits from reduced emissions of other hazardous substances.

The conditions under which high manganese exposures might occur are not specified here. It is simply assumed that there exist conditions at which high exposures occur to an individual or to some small subgroup of the population. Refined analysis of manganese exposures based on the SCREAM model [results of the SCREAM analysis were presented at the EPA manganese/MMT workshop by Gerry Anderson of Systems Applications] and on measured lead exposures [results presented at the workshop by

Ralph Roberson of Systems Applications] suggest that it is extremely unlikely that significant numbers of people would be exposed at levels approaching the RfC for manganese.

The risk estimates for the hypothetical high exposure group assume exposure to auto emissions in sufficiently high concentrations to produce an exposure to manganese, with MMT use, at the level of the manganese RfC (of 0.4 $\mu g/m^3$). This assumption is adopted solely for the purposes of the present analysis; it does not indicate that such exposures will actually occur. Rather, calculations based on exposure at the level of the manganese RfC simply provide a useful point of comparison. Background exposures to manganese are included. Since the high exposure group experiences exposures to manganese of 0.4 $\mu g/m^3$ and 0.04 $\mu g/m^3$ is background, 0.36 $\mu g/m^3$ is due to auto emissions.

The analysis assumes that exposures to auto emissions of manganese, CO, NO_x, particulates, formaldehyde, benzene, acetaldehyde, and 1,3 butadiene occur in proportion to emissions of each substance. Other sources (i.e., background and other non-auto sources) are then included to get total exposure. Background levels were included for manganese, CO, NO_x, and particulates; all exposures to the four hydrocarbons were assumed to come only from autos.

Data

A large quantity of data are available regarding emissions of hydrocarbons, CO and NO_x for vehicles using unleaded gasoline containing MMT in comparison to vehicles running on the same fuel without MMT. The data come from a test fleet of 48 cars (6 cars of each of 8 models, 3 for each fuel), run for 75,000 miles. This data is used as the basis for computed CO and NO_x emissions with and without MMT.

The results from the fleet tests are given in Table 1. Data for the first 1,000 miles are excluded because all cars were run on clear fuel during this interval.

Table 1

Emissions Data (gm/mile) from Fleet Tests

Data from 1,000-75,000 miles

	Clear	MMT	Change
HC	0.289	0.307	6.23%
CO	3.30	3.08	-6.67%
NO_x	0.55	0.43	-21.82%

As Table 1 indicates, CO and NO_x emissions were lower with fuel containing MMT than with the clear fuel, but hydrocarbon emissions were higher with MMT. Because the fuel with MMT has higher octane than the comparison clear fuel, the observed emission rates may differ from what would be experienced with actual MMT use, since MMT would

likely be used to provide replacement fuel of equivalent octane. Additives used to raise octane such as aromatics also typically increase emissions, particularly emissions of hydrocarbons. As a consequence, the fleet data probably understate the degree of emissions reduction that would be obtained from MMT use.

An additional difference observed between the emissions with and without MMT use is the reactivity of the mix of hydrocarbons. The reactivity was lower with MMT use by 16% - 20% (see waiver application). Because the reactivity of hydrocarbons is a measure of ozone-forming potential, these data indicate that emissions for cars with MMT use would lead to lower ozone levels than would emissions from the same fleet using unleaded fuel without MMT.

Significantly less data are available for the four carcinogens of concern. Emission measurements of many hydrocarbon species were made in one pair of test cars, Ford Crown Victorias, at 67,000 miles. Three different pairs of fuels were used: Howell EEE, commercial unleaded, and reformulated gasoline, each with and without MMT. Unlike the fleet tests, octane levels with and without MMT were matched through addition of xylenes to the fuel that did not contain MMT. The results of these tests are indicated on Figure 1.

Two aspects of these data are important. First, emissions of the four carcinogens were significantly lower with MMT use than were emissions of total hydrocarbons. This

indicates that use of MMT apparently produces a less hazardous mix of hydrocarbons (based on carcinogenic risk) in comparison to fuels with xylenes added to equilibrate octane. For the reformulated gasoline tests, total HC emissions were slightly higher with MMT (by about a percent), but emissions of the individual carcinogens were lower with MMT by between 7 to 12%. Second, reductions of hydrocarbon and carcinogen emissions were substantially greater with Howell EEE and commercial gasoline than with the reformulated gasoline.

In this analysis, estimates of emission reductions for the four carcinogens are based on the average for all three gasolines. This is likely to underestimate risk reduction benefits from MMT use for areas where reformulated gasolines are not used.

One issue that bears examination is the degree to which the Ford Crown Victoria data are representative of the fleet as a whole. A way to examine this question is to look at how the Ford Crown Victorias performed in the fleet tests in comparison to the fleet average. Table 2 gives the performance data for the Ford Crown Victorias for both the 75,000 mile fleet tests and for the speciation tests at 67,000 miles. As the table notes, the fleet tests involved fuels unmatched in octane; the speciation tests used fuels of equal octane.

A comparison of the top half of Table 2 with Table 1 indicates that the Ford Crown Victoria has higher emissions of HC and NO_x than the fleet as a whole for both MMT

Table 2

Emissions Data (gm/mile) from Ford Crown Victorias

Data from 1,000-75,000 miles, octane not matched

	Clear	MMT	Change
HC	0.510	0.516	1.18%
CO	1.95	1.20	-38.46%
NO_x	1.03	0.78	-24.27%

Data at 67,000 miles, octane matched

	Clear	MMT	Change
HC	0.568	0.513	-9.71%
CO	2.21	1.38	-37.50%
NO_x	1.37	0.94	-31.53%

and clear fuel; this is not surprising for a large car. The relative changes in hydrocarbon emissions between MMT and clear fuel are typical of those observed for the fleet. Hydrocarbon emissions were 6.23% higher with MMT for the full fleet; they were 1.18% higher in the Ford Crown Victorias. The bottom part of Table 2 indicates that when emissions are compared for fuels of equal octane, where xylenes were added to the clear fuel, HC emissions from the clear fuel increased by about 11%.

For the purpose of estimating reductions of the four carcinogens, the Ford Crown Victoria measurements are roughly consistent with the fleet data in terms of the effect of MMT on hydrocarbon emissions; the fleet tests found HC emissions to be 6.23% higher with MMT, in the same test and with the same fuels, the Ford Crown Victoria HC

emissions were 1.18% higher with MMT use. The Ford Crown Victoria speciation data are the basis for estimates of emission reductions with MMT use for the four carcinogens.

Analytical Results

- **Carcinogens**

The midpoints of the ranges estimated by Adler and Carey for emissions and exposures are given in Table 3, along with unit risk factors and risks. A unit risk factor is the estimated lifetime risk of getting cancer from exposure to a substance at a concentration of 1 $\mu g/m^3$. The individual risk column refers to the plausible upper bound risk for 70 years exposure. Cancer cases per year, often referred to as the population risk, are calculated by dividing individual risk by 70 to get annual individual risk, and then by multiplying by 260 million, the estimated 1995 population. Several decimal places are shown; these estimates are highly uncertain and high precision should not be inferred by the number of decimal places.

Taking the average reductions in carcinogen emissions for the three fuels tested (see Figure 1), the average individual lifetime risk from exposures to the four carcinogens would be 5.8×10^{-5} with MMT use; this is 1.29×10^{-5} lower than for the case where MMT is not used. For the U.S. population of 260 million in 1995, the cancer risk

reduction corresponds to 48 cases of cancer per year. These results are shown in Table 4 and on Figures 2 and 3. The columns may not add exactly due to rounding.

Table 3

Carcinogen Emissions and Risks, 1995

Average Exposures based on Adler and Carey (1989)

(Assumes no MMT Use)

	Emis gm/mile	Conc $\mu g/m^3$	Risk factor	Indiv risk	Cancer cases/yr
Formaldehyde	0.0155	0.54	1.3E-5	7.02E-6	26.1
Benzene	0.0575	2.6	8.3E-6	2.16E-5	80.2
Acetaldehyde	0.0045	0.155	2.2E-6	3.41E-7	1.3
1,3 Butadiene	0.0045	0.150	2.8E-4	4.2 E-5	156.0
Sum of 4 HCs				7.09E-5	263.5

Table 4

Carcinogen Risks

Average Exposure Risks with and without MMT

	Without MMT		With MMT	
	Indiv risk	Cancer cases/yr	Indiv risk	Cancer cases/yr
Formaldehyde	7.02E-6	26.1	5.98E-6	22.2
Benzene	2.16E-5	80.2	1.62E-5	60.1
Acetaldehyde	3.41E-7	1.3	2.76E-7	1.0
1,3 Butadiene	4.2 E-5	156.0	3.56E-5	132.1
Sum of 4 HCs	7.09E-5	263.5	5.80E-5	215.5

With MMT use at 1/32 gram manganese per gallon, with 20 miles per gallon and a 20% emissions factor, the average manganese emission rate is 0.000313 grams manganese per mile. Assuming that emissions (in gm/mile) multiplied by 35 gives average exposure in $\mu g/m^3$, then average manganese exposure due to MMT use in unleaded gasoline would be 0.0109 $\mu g/m^3$. This estimate is consistent with the ORD exposure analysis.

For the high exposure case, i.e., for exposures at high concentration where manganese concentrations are at the RfC, the manganese concentration is 0.4 $\mu g/m^3$, of which 0.04 $\mu g/m^3$ is due to background and 0.36 $\mu g/m^3$ to auto emissions. The concentration of auto emissions in this environment is 0.36/0.0109 times average levels, or 32.9 times the average exposure level. This factor of 32.9 is used to calculate concentrations of the four carcinogens and CO, NO_x, and particulates in the high exposure case.

It merits noting that the SCREAM and other exposure modeling based on historical lead usage in gasoline did not find any group with average exposures approaching this concentration. For example, exposures at 0.36 $\mu g/m^3$ are beyond the tail of the SCREAM exposure distribution.

For anyone exposed at exactly the manganese RfC, the risk from exposures to the four carcinogens is given in Table 5. (The average exposure concentration in Table 3 was multiplied by 32.9 in order to obtain the high exposure concentration for each carcinogen.) The third column of this table indicates the calculated annual cancer

incidence per million population at this high exposure level. This measure (cancer incidence per million per year) is given as a benchmark and is not meant to indicate that there could be a million people exposed at this level. [Just as it is appropriate to measure travel in miles per hour, even for trips of less than one mile or one hour, so too can risk to a small population be described in terms of incidence per million per year.]

Table 5

Carcinogen Exposures and Risks

High Exposure Environment

(Without MMT Use)

	Conc $\mu g/m^3$	Indiv risk	Cancer cases/yr per 10^6
Formaldehyde	17.77	2.31E-4	3.3
Benzene	85.58	7.10E-4	10.1
Acetaldehyde	5.1	1.12E-5	0.16
1,3 Butadiene	4.94	1.38E-3	19.7
Sum of 4 HCs		2.33E-3	33.4

Again, risk reductions associated with MMT use for the high exposure group were calculated based on the measured emission reductions for the four carcinogens presented in Figure 1. The results of this calculation are given in Table 6 and on Figures 4 and 5. For anyone exposed at the high levels used in these calculations, the plausible upper bound lifetime individual cancer risk is estimated at 2.33×10^{-3} without MMT use and

1.9 x 10^{-3} with MMT. The reduction in lifetime cancer risk with MMT use, as indicated in Table 7, is calculated to be greater than 4 x 10^{-4}.

Table 6

Carcinogen Risks

High Exposure Environment with and without MMT

	Without MMT		With MMT	
	Indiv risk	Cancer cases/yr per 10^6	Indiv risk	Cancer cases/yr per 10^6
Formaldehyde	2.31E-4	3.3	1.97E-4	2.8
Benzene	7.10E-4	10.1	5.33E-4	7.6
Acetaldehyde	1.12E-5	0.16	9.07E-6	0.13
1,3 Butadiene	1.38E-3	19.7	1.17E-3	16.7
Sum of 4 HCs	2.33E-3	33.4	1.90E-3	27.3

Table 7

Carcinogen Risks

High Exposure Environment Risk Reductions with MMT

	Indiv risk	Cancer cases/yr per 10^6
Formaldehyde	3.41E-05	0.49
Benzene	1.78E-04	2.54
Acetaldehyde	2.14E-06	0.03
1,3 Butadiene	2.12E-04	3.03
Sum of 4 HCs	4.26E-04	6.08

In addition to their carcinogenic risks, formaldehyde, benzene, and acetaldehyde are also hazardous based on non-carcinogenic risk endpoints. California's Department of Health Services (CDHS) has set reference concentration values for these substances for use in risk assessments for incinerator emissions. These reference standards (equivalent to RfCs) are based on chronic non-cancer endpoints. The established values are 3.6 $\mu g/m^3$ for formaldehyde, 71 $\mu g/m^3$ for benzene, and 40 $\mu g/m^3$ for acetaldehyde, and 1 $\mu g/m^3$ for manganese.

Figure 6 indicates the significance of exposures to manganese in comparison to benzene and formaldehyde based on chronic non-cancer effects. Unlike the previous figures, the reference or RfC value for manganese on this figure is the California value noted above of 1 $\mu g/m^3$, 2.5 times higher than the EPA RfC. This value is used because it is presumably consistent in its derivation and protection objectives with the exposure concentration reference value derived by CDHS for formaldehyde, benzene, and acetaldehyde. The significant point here is that for exposure environments in which the California acceptable exposure standard for manganese is experienced, formaldehyde exposures are calculated to occur at over twelve times its acceptable exposure standard. (This ratio was obtained by comparing the California reference standard of 3.6 $\mu g/m^3$ to the high exposure concentration of formaldehyde (17.77 $\mu g/m^3$; see Table 5) after it has been multiplied by the factor of 2.5.) Use of MMT would reduce formaldehyde exposures to about ten and one half times acceptable levels. While this is still a high

exposure, it reflects a reduction of almost two times the California acceptable exposure standard.

- **Noncarcinogenic Emissions**

The major noncarcinogenic emissions of concern from gasoline-powered autos are CO and NO_x. Although particulates are not a significant regulatory issue for gasoline-powered vehicles, a slight increase in emissions of particulates with MMT use were measured in tests sponsored by Ethyl. The significance of these emissions are assessed below.

As was noted above, in the fleet tests, vehicles using fuel containing MMT had lower emission of both CO and NO_x; CO was reduced by 6.67% and NO_x by 21.82%. In its 1990 waiver application, Ethyl provided an analysis that these observed CO and NO_x reductions would correspond to annual reductions in emissions of 985 million pounds for CO and 633 million pounds for NO_x in 1999, against manganese emissions of 1.5 million pounds per year, assuming a 20% emissions rate. Other factors used for calculating emissions and emission reductions for CO and NO_x are based on data in National Air Quality and Emissions Trends Report, 1987, EPA OAQPS, March 1989.

- **Particulates**

In recent tests conducted at the Southwest Research Institute for Ethyl, particulate emissions were measured to be 0.003 to 0.004 gm/mile higher on average with MMT than without. For purposes of calculation, particulate emissions are taken to be 0.008 gm/mile without MMT and 0.012 gm/mile with MMT. This increase in particulate emissions of 0.004 gm/mile produces an average increase in ambient particulate levels of around 0.14 $\mu g/m^3$, based on the rule that emissions in gm/mile multiplied by 35 gives average concentration in $\mu g/m^3$.

For a high exposure micro-environment, concentrations were calculated above to be 32.9 times higher than ambient average; for particulates, this corresponds to an increase in the high exposure micro-environment concentration of particulates of 4.6 $\mu g/m^3$ for the case where all unleaded gasoline contains MMT, in comparison to no MMT use. The particulate concentration in such a micro-environment, if all unleaded gasoline contained MMT, would be around 14 $\mu g/m^3$. For purposes of comparison, the annual National Ambient Air Quality Standard for total suspended particulates (TSP) is 75 $\mu g/m^3$; for PM_{10}, the annual standard is 50 $\mu g/m^3$. The average U.S. level of TSP was around 50 $\mu g/m^3$ in 1987. So for the hypothetical high-exposure micro-environment in which the manganese concentration reaches the level of the RfC, particulate concentrations due to emissions from autos using unleaded gasoline with MMT only reach about one fourth the level of the standard. The incremental increase in the high exposure environment due to

MMT is less than one tenth the level of the ambient standard for TSP. As another basis for considering the magnitude of these emissions, particulate emissions would increase by about 6,000 metric tons per year with MMT use, based on 120 million autos driven 12,000 miles per year. Particulate emissions in 1987 were 7 million metric tons, of which 1.4 million were from transportation.

Table 8 gives emissions rates for CO, NO_x, particulates and manganese. The estimates assume low emissions of CO and NO_x; this leads to correspondingly conservative projected reductions in CO and NO_x due to MMT use.

Table 8

1999 Emissions of CO, NO_x, Particulates and Manganese

(grams/mile)

	Without MMT	With MMT	Difference
CO	3.08	2.87	-0.205
NO_x	0.604	0.473	-0.132
Particulates	0.008	0.012	0.004
Manganese	0.0	0.000313	0.000313

Table 9 gives the calculated average concentrations of these substances, including contributions from non-automotive sources. The "Ratio" refers to the ratio of calculated concentrations to regulatory standards. For CO, the ratio refers to the eight-hour standard of 9 ppm; for NO_x and particulates, the ratio is based on the annual ambient

standard (0.053 ppm for NO_x, 75 $\mu g/m^3$ for particulates); for manganese the ratio is based on the RfC of 0.4 $\mu g/m^3$.

Table 9 uses two exposure cases, average and high, consistent with the approach used in the carcinogen analysis. Unlike the calculation for carcinogens, non-automotive sources are accounted for here. As for the carcinogens, the ratio of auto emissions (in gm/mile) to exposure (in $\mu g/m^3$) is assumed to be 1:35.

Table 9

Concentrations of CO, NO_x, Particulates and Manganese, 1999

($\mu g/m^3$)

	Auto contrib	Other	Total	Ratio
Average Exposure Case				
CO (without MMT)	107	228	335	0.033
CO (with MMT)	100	228	328	0.032
NO_x (without MMT)	21	20	41	0.415
NO_x (with MMT)	16.5	20	37	0.369
Particulates (w/out MMT)	0.28	50	50	0.667
Particulates (with MMT)	0.42	50	50	0.672
Manganese (without MMT)	0	0.04	0.04	0.100
Manganese (with MMT)	0.01	0.04	0.05	0.125
High Exposure Case				
CO (without MMT)	3,544	228	3,772	0.368
CO (with MMT)	3,307	228	3,535	0.345
NO_x (without MMT)	696	20	716	7.187
NO_x (with MMT)	544	20	565	5.663
Particulates (w/out MMT)	9.2	50	59.2	0.789
Particulates (with MMT)	13.8	50	63.8	0.850
Manganese (without MMT)	0	0.04	0.04	0.100
Manganese (with MMT)	0.36	0.04	0.4	1.000

Analytical Uncertainties

The health risks associated with all exposures examined in this analysis are highly uncertain. EPA characterizes cancer risk estimates based on EPA-developed methods as plausible upper bounds; actual risks are not known and could be zero. Similarly, risks at an RfC level are not known and could be zero. RfCs are derived such that exposures at the RfC constitute virtually safe doses; such exposures should not pose a significant risk.

A number of uncertainties can be noted: the average level of manganese emitted is not known precisely, the speciation data on which calculated reductions of carcinogenic emissions are based are limited, and the future mix of gasoline, e.g., use of reformulated gasoline with or without MMT versus commercial unleaded, is uncertain.

In this analysis, as in risk analysis generally, the uncertainties in health risk are significantly greater than those in exposure. Often the uncertainty in exposure can be quantified. The ranges given by Adler and Carey in their analysis of cancer risks from auto emissions reflect uncertainties in exposure, but not in health risk.

Uncertainty bars are not provided here, on either the figures or tables, because it is not possible to do so in a meaningful way. For the cancer risk calculations, the bar graphs are uncertainty bounds; the actual risks may range from zero to the plausible upper bound (and there is some remote chance that actual risks could be even higher). For

non-cancer risks, one could perhaps estimate the uncertainties in exposure and describe them in relation to the relevant exposure standard. But because the absolute risks associated with exposures calculated here are not known, calculating uncertainties due to exposure alone would give a false sense of precision.

Often, as is the case here, a risk assessment that is highly uncertain regarding absolute levels of risk can provide a helpful comparison of relative risks, calculated on an equivalent basis. This is why EPA risk assessment methods and reference levels were used in this paper to the extent that they existed. The objective here is to calculate whether, based on currently available data and using consistent methods to calculate risks, MMT leads to risk reductions or risk additions.

Other Benefits and Risks

Table 10 lists significant or potentially significant effects from widespread MMT use. Many values in the table were taken from Appendix 7 of the 1990 Ethyl submission, "Total Pollutant Reduction," and from other submitted documents. The assumptions behind this table are described in this paper and in Appendix 7; emission reduction estimates are based on 100% use of MMT in unleaded gasoline and on a 20% manganese emission factor.

Table 10

Environmental and Social Benefits and Risks from MMT Use	
Environmental Benefits	Environmental Risks
Auto Emission Reductions CO - 985,000,000 lbs/year NO_x - 633,000,000 lbs/year Aromatics - 32,000,000 lbs/year Formaldehyde - 3,500,000 lbs/year Benzene - 3,500,000 lbs/year HC - uncertain; likely to be some reduction based on comparison with fuel of equal octane. Reactivity of MMT HC lower by 19-25%, so ozone reduced Refinery Emission Reductions NO_x - 11,000,000 lbs/year CO - 3,000,000 lbs/year Particulates - 1,100,000 lbs/year SO_x - 150,000 lbs/year CO_2 - 10,000,000,000 lbs/year Import Oil Reductions 30,000,000 bbls/year (worth over $600,000,000 per year) Lower cost to produce unleaded gasoline MMT improves octane at about 1/3 the cost of competing methods Potentially beneficial effect to agriculture	Auto Emission Increases Mn - 1,500,000 lbs/year (assumes 20% emissions) Particulates - 13,000,000 lbs per year Impacts from MMT ground water or direct exposure

The value of fuel saved through reduced refining energy requirements was put at $600 million per year (for $20/bbb oil); arguably this is the most significant item on the list. The reduction of U.S. oil imports -- 30 million barrels per year -- is equivalent to 4 days of imports annually. The war in the Persian Gulf leaves no doubt that there are risks associated with acquiring oil imports that extend beyond their effect on balance of trade. Perhaps the fact that MMT will let us reduce imports while also reducing costs is potentially its most important national benefit.

While the reduction in CO_2 emissions, due to reduced fuel use in refining, is small in comparison to total U.S. emissions, most options for reducing CO_2 emissions in the short term have small effects in comparison to total emissions, and many of these other options for reducing CO_2 emissions are expensive. Conversely, the CO_2 reductions associated with MMT use are accompanied with cost savings to refiners of unleaded gasoline.

Table 10 also notes that the reactivity of hydrocarbon emissions was found to be significantly lowered with the addition of MMT. This is of great potential significance because hydrocarbon reactivity is taken to be a measure of ozone-forming potential.

Discussion of Results

All analyses to date find that ambient levels of manganese would remain close to background levels and that average exposures to manganese would remain well below the

RfC. Consequently, health and environmental consequences from manganese emissions to the country as a whole are insignificant. In contrast, benefits in energy savings and environmental quality are significant.

The trade-off, when viewed from a national perspective, is whether the possible risks from manganese emissions from MMT use outweigh the potential benefits from its use. The potential benefits include reductions in exposures to CO, NO_x, and ozone. In addition, the reduction in cancer risks associated with lower aromatic content and emissions for MMT fuel, is estimated here to be up to 50 cases per year. The national perspective on the risks and benefits of MMT use is provided by Table 10.

But the national perspective is not the appropriate perspective from which to consider exposures to highly exposed individuals. The issue, considered from the perspective of a parking garage attendant or other highly exposed individual, is whether use of MMT would lead to an increase or decrease in risk, taking all emissions into account. As noted here, exposure assessments indicate that few, if any, people would be exposed at levels approaching that of the RfC. And it is important to understand that exposures to auto emissions at levels so high that they approach the manganese RfC would also lead to high exposures to carcinogens. The upper end of individual lifetime risk levels typically found acceptable by EPA is roughly 10^{-6} to 10^{-4} (see articles by Milvy, Travis, and Lave and Byrd in De Minimis Risk, Whipple, ed., Plenum Press 1987). For a person in this high exposure group, a reduction in exposures to these aromatics due to MMT use

corresponds to a cancer risk reduction of about 4×10^{-4}. This should outweigh the concern of manganese exposure at the RfC level, since exposures at the RfC level are, as defined by EPA, "likely to be without appreciable risk of deleterious effects during a lifetime." In addition to the risk reductions due to aromatics, a reduction in NO_x exposure from levels well above the ambient standard may be more significant in terms of health risk than the manganese exposure.

Figure 7 provides an overall perspective on the trade-offs faced by someone at high exposure to auto emissions. The logarithmic scale compresses the significant risk reductions provided by MMT. Note that this figure indicates the results for MMT use for both carcinogens and non-carcinogens. The left and right scales are not directly comparable and it should not be inferred that the risks of the carcinogens and non-carcinogens are comparable. The scales were selected so that a lifetime cancer risk of 1×10^{-6} corresponds to an exposure at the RfC; this was because these levels have traditionally been taken by EPA to represent safe levels.

This analysis indicates that a car run on unleaded gasoline with MMT has a less harmful mix of emissions than does a comparable car run on unleaded gasoline of equivalent octane. Whether one compares these two cases based on annual emissions in the U.S. or on the basis of potential exposures in high-concentration micro-environments, the analysis of net risk indicates that MMT use in unleaded gasoline would result in a net public health benefit.

References

Waiver application for the use of HiTEC®3000 in unleaded gasoline, Ethyl Corporation, submitted on May 9, 1990 to EPA's Office of Mobile Sources.

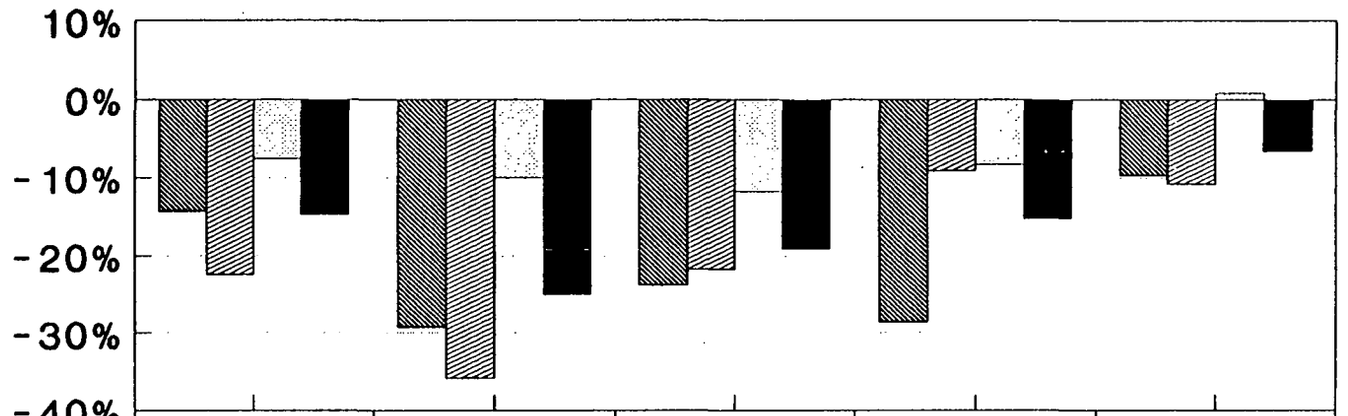
Adler, Jonathan M. and Carey, Penny M., US EPA Ann Arbor, "Air Toxics Emissions and Health Risks from Mobile Sources," Air and Waste Management Association Paper 89-34A.6, 82nd Annual Meeting & Exhibition, Anaheim, CA, June 25-30, 1989. This analysis is an update and extension of 1987 and 1988 papers by Carey and Somers.

EPA Office of Air Quality Planning and Standards, Monitoring and Reports Branch, National Air Quality and Emissions Trends Report, 1987 EPA-450/4-89-001, March 1989

California Air Resources Board based on California Department of Health Services exposure levels. January 1991 revisions to Appendix F of Health Risk Assessment Guidelines for Nonhazardous Waste Incinerators, August 1990,

Whipple, Chris G., editor, De Minimis Risk, Society for Risk Analysis, Contemporary Issues in Risk Analysis, Volume 2, Plenum Press 1987.

Emissions with HiTEC 3000 Compared to Other Fuels of Equal Octane



	Formaldehyde	Benzene	Acetaldehyde	1,3 Butadiene	Total HC
Howell EEE	-14.3%	-29.2%	-23.8%	-28.6%	-9.7%
Commercial	-22.4%	-35.8%	-21.7%	-9.1%	-10.8%
Reformulated	-7.6%	-10%	-11.8%	-8.3%	0.8%
Average	-14.7%	-25%	-19.1%	-15.3%	-6.6%

Figure 1

Risks from Auto Emissions
- Average Exposures -

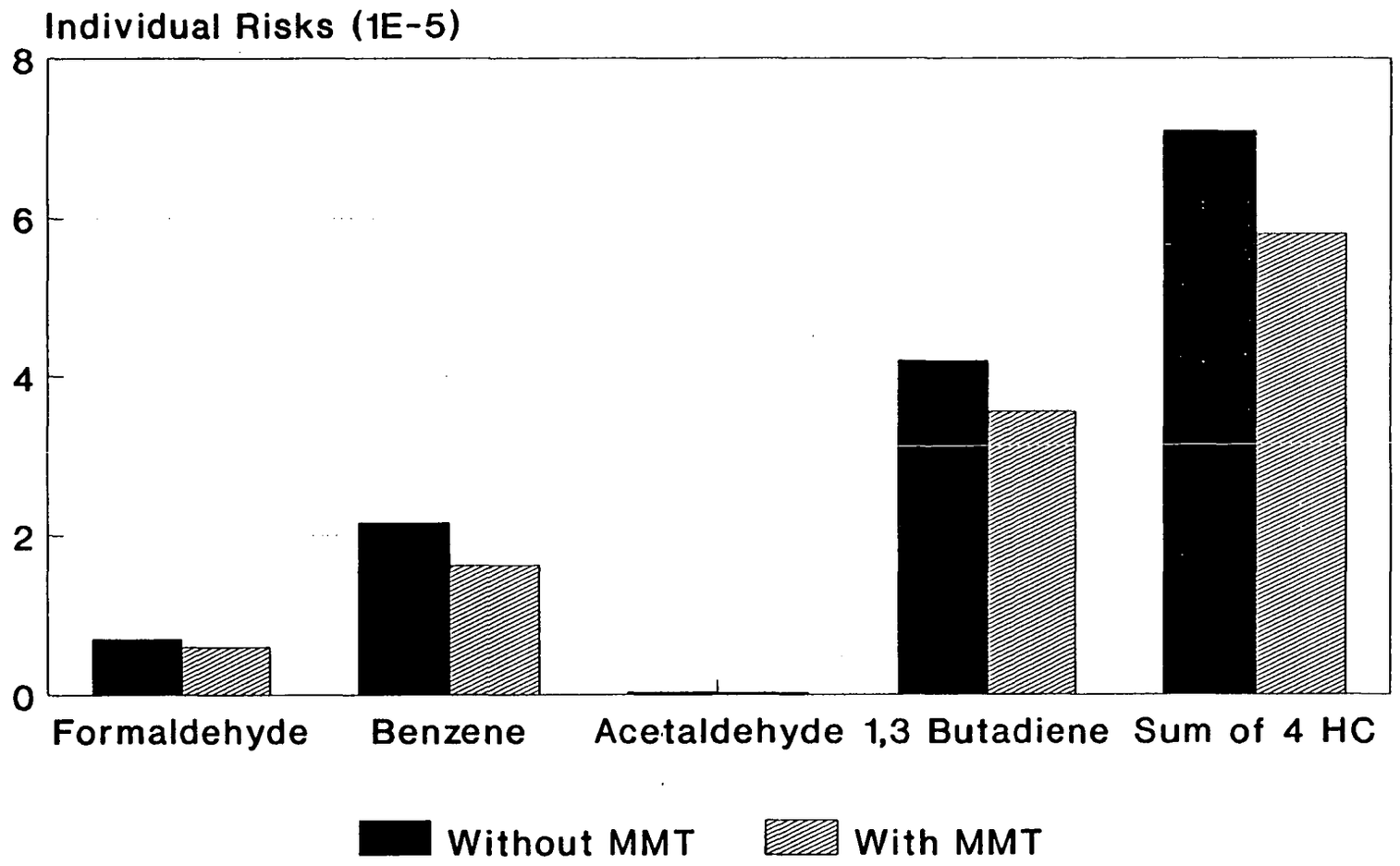


Figure 2

Nationwide Risks from Auto Emissions
Based on Adler & Carey Estimate for 1995

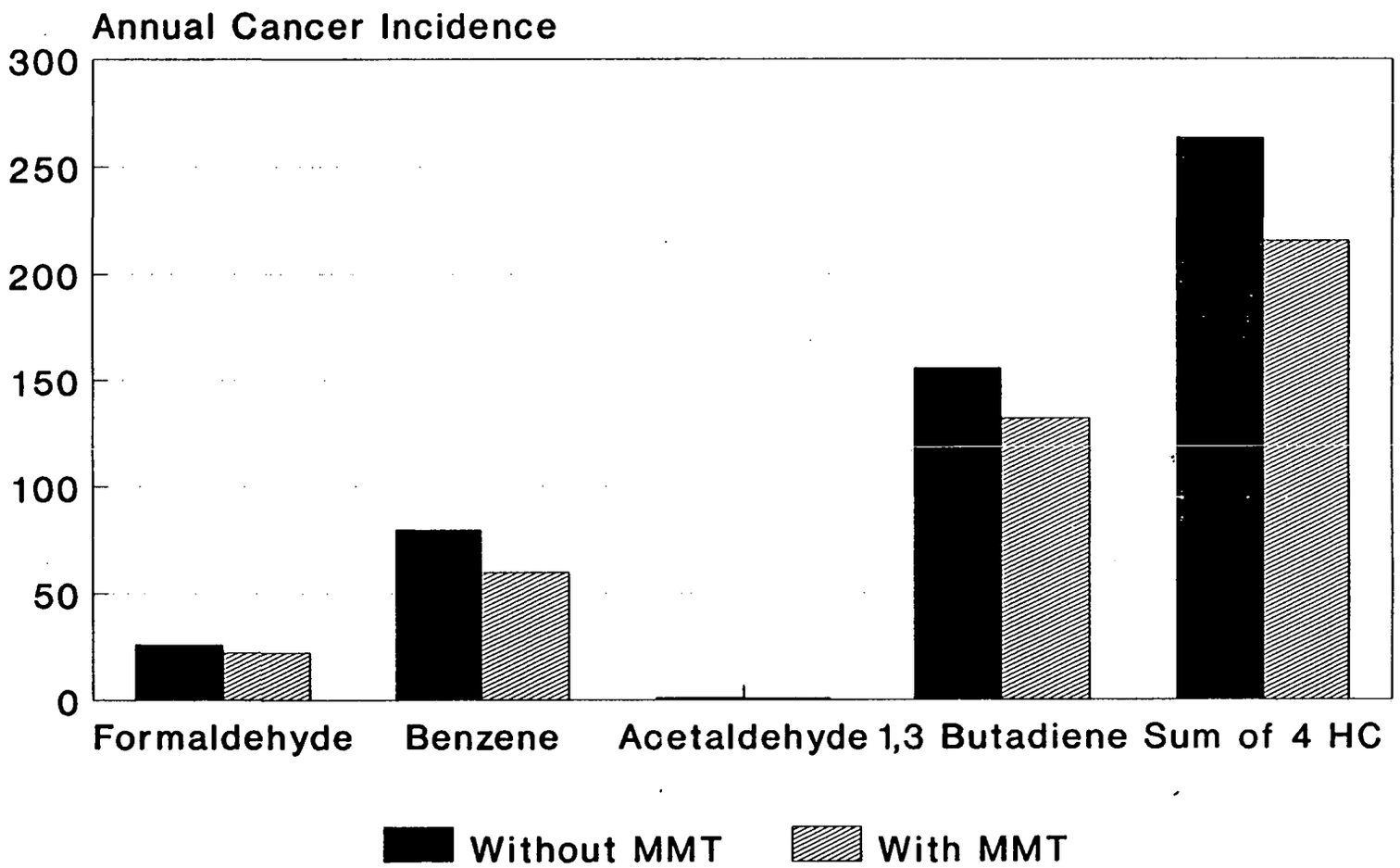


Figure 3

Risks from Auto Emissions
- Exposures when Mn at the RfC -

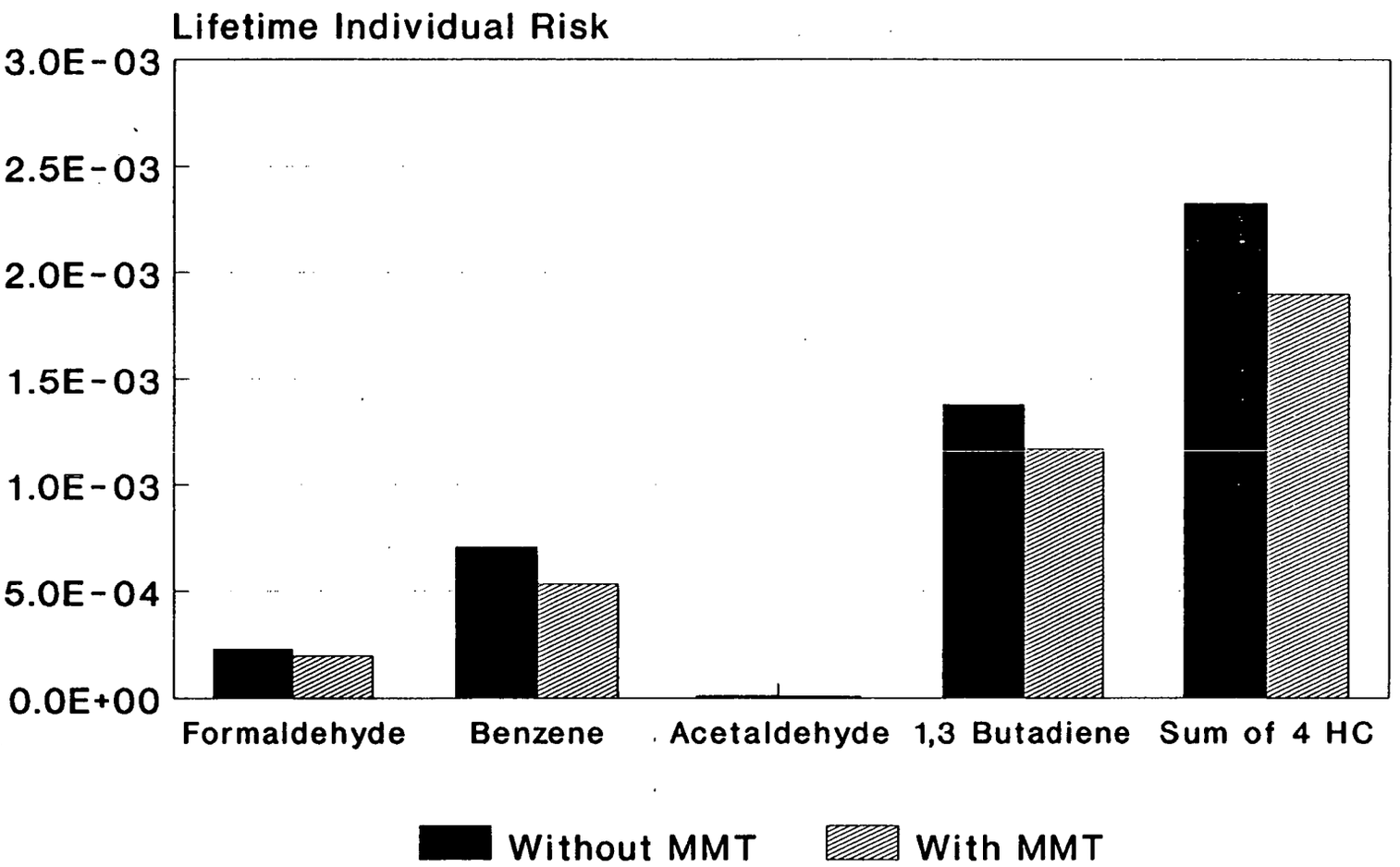


Figure 4

Cancer Risks to High Exposure Population
Assumes Exposures at Manganese RfC

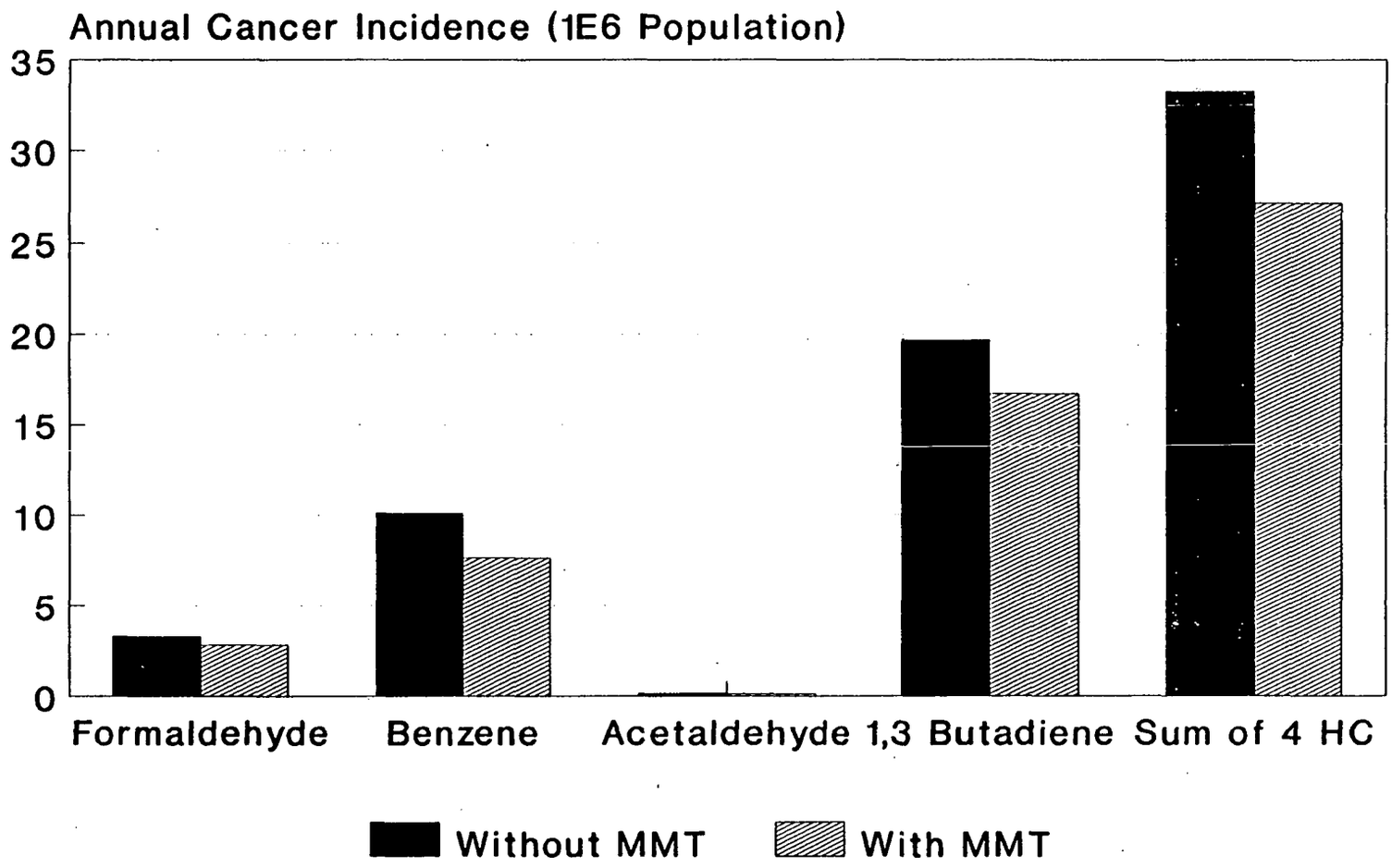


Figure 5

Noncancer Concerns with HC Exposures - Microenvironment at CDHS Ref. Level -

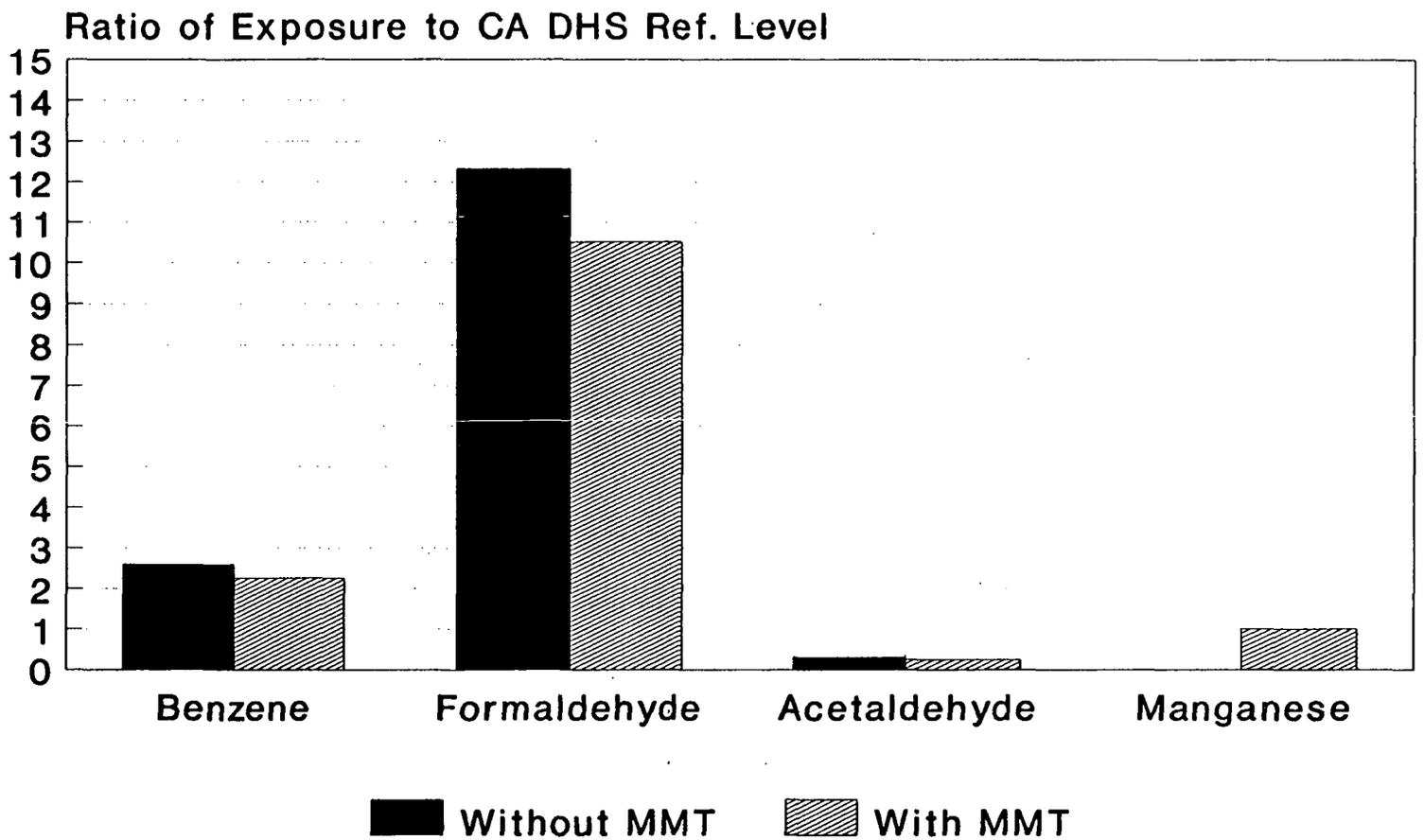


Figure 6

Risks from Auto Emissions
- Micro-environment at Mn RfC -

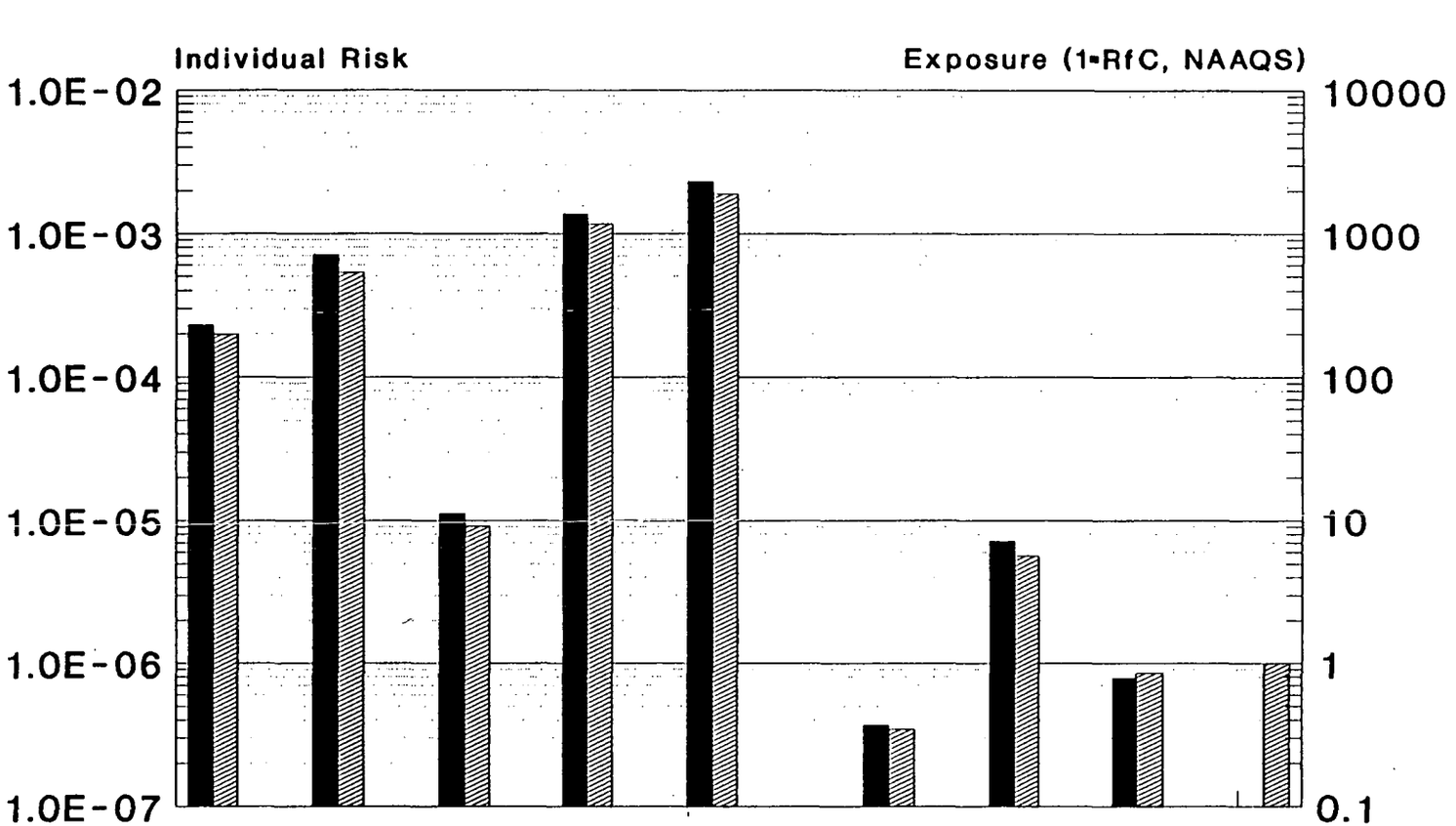


Figure 7

PART I

REVIEW OF INFORMATION ON MANGANESE AND THE OXIDATION PRODUCTS OF MMT COMBUSTION

REPORT PREPARED

BY

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FOR

HEALTH AND WELFARE CANADA

1988

A REVIEW OF INFORMATION ON MMT AND MANGANESE OXIDES

A.1 Properties and analysis

A.1.1 Physical and chemical properties

A.1.1.1 **MMT** The physical and chemical properties of commercial grade methylcyclopentadienyl manganese tricarbonyl (MMT; also known as MCMT or MCPT) have been described by Hinderer (1). MMT is a dark orange liquid which is completely soluble in hydrocarbons and slightly soluble in water (i.e., 70 mg/L at 25°C). The pure compound has a molecular weight of 218.1 g ($C_9H_7O_3Mn$), and contains 25.2% manganese by weight; the commercial grade has a slightly lower manganese content of 24.4%. At 20°C, pure MMT has a density of 1.38 g/mL and a viscosity of 0.0052 Pa.s. It has a flash point of 110°C, a freezing point of -2.2°C, a boiling point of 232.8°C, a vapour pressure of 5.87 Pa at 0°C, and a half-life, in air, of between 8 and 18 seconds (2). According to Ter Haar et al. (2), the decomposition involves both light and oxygen; the wavelengths of light, lying between 340 and 440 nm, represent the active zone. The products of this photolytic decomposition are manganese oxides and carbonates. Other data on MMT are given in Meek and Bogoroch (3), and in Jaques (4).

A.1.1.2 **CMT** The physical and chemical properties of cyclopentadienyl manganese tricarbonyl (CMT), the non-methylated parent compound of MMT, have been described by the Ethyl Corporation (5). It has a molecular weight of 204.1 g ($C_8H_5O_3Mn$), and contains 26.9% manganese by weight.

A.1.1.3 **Manganese oxides** The main decomposition product, resulting from either combustion or photolysis of MMT/CMT, is manganese (II, III) oxide, Mn_3O_4, M.Wt. 228.8 g; trace amounts of manganese (III) sesquioxide, Mn_2O_3 (M.Wt. 157.87 g), are also formed (6). These mixed oxides, in which manganese exhibits the oxidation states of 2 and 3, are formed when most other oxides of manganese are ignited in air. Thus, although manganese (II) oxide and manganese (IV) oxide could initially be formed from MMT, they will, under the combustion conditions prevailing in automobile engines, nearly all be converted into the Mn_3O_4, which usually has a brownish-black colour, and has a particulate appearance. The latter is insoluble in water, and has a density of 4.9 kg/m^3.

A.1.2 Analytical methods

A.1.2.1 **MMT and CMT** The analysis of MMT and CMT is usually carried out via an atomic absorption spectrophotometric (AAS) determination of manganese (3; 7; 8; 9); in some cases (1), the infra-red (IR) absorption of pentane extracts at 1935 cm^{-1} has also been used.

A.1.2.2 **Manganese oxides and inorganic manganese** Until recently, the most common analytical technique was flame-AAS, with or without Zeeman effect background correction (10; 11; 12; 13). However, as the concentration of manganese is often at the ng/mL concentration level, flame-AAS is now being replaced by electrothermal-AAS, the latter being 100-1000 times more sensitive (14). Other common methods of analysis (e.g., neutron activation

analysis and X-ray fluorescence) are summarized in the World Health Organization (WHO) report on manganese (15). Particle sizing, when performed, is sometimes carried out by scanning electron microscopy (SEM) (16), or, more usually, by some form of an aerosol particle monitoring device (7), often a cascade impactor (17; 18).

A.2 Sources of manganese in the environment

A.2.1 **Natural occurrence** In this section, all manganese sources, contributing to environmental concentration levels, will be considered in order to place the contribution of MMT-derived manganese into perspective.

A.2.1.1 **Soil** Manganese, the twelfth most abundant element in the earth's crust, is present in over 100 naturally occurring minerals; these are commonly hydroxides, sulphides, carbonates, silicates, and oxides, the latter being important ore minerals. The average concentration of manganese in the earth's crust is 1000 ppm, and ranges from 0 to 7000 ppm (19); erosion, leaching and biological processes disseminate Mn throughout the soil, in which concentration levels are usually around 800 ppm (see section A.3.4).

Manganese is present, at the earth's surface, in the II, III and IV oxydation states, but only Mn (II) and Mn (IV) are stable in soil solution. Manganese (II) is predominant in about 70-90% of cases at pH's below 7 (20); in alkaline and neutral soils, Mn exists primarily in solid forms, principally as oxyhydroxides of mixed valence. Up to 38% of the total manganese in surface soils is reducible oxyhydroxides; these tend to accumulate in well-oxidized soils, solubilities increasing under reducing conditions. Small amounts of manganese form Mn(II)[SO_4] complexes at low pH.

The chemical activity of manganese in water is dependent on both pH and Eh. Pyrolusite (MnO_2) is the most stable common specie, across a wide range of Eh and pH; its dissociation to ionic Mn (II) can be expressed as:

$$MnO_2 + 4H^+ + 2e^- = Mn^{2+} + 2H_2O$$

which leads to the following relationship:

$$Eh = 1.208 - 0.059 [\ 2pH - 1/2pMn\ ]$$

However, this equation is incapable of correctly predicting the concentration levels of manganese (II) in soils; alternative relationships have been sought, including equations derived from the dissolution of manganese sesquioxide:

$$Mn_2O_3 + 6H^+ + 2e^- = 2Mn^{2+} + 3H_2O$$

Radioactive tracer studies have shown that equilibrium between ^{54}Mn and soil Mn can take from 0.5 to 80 hours before being established, depending on the soil type.

A.2.1.2 **Ocean sediments and seawater** Ocean sediments often contain manganese oxide nodules, frequently impacted to form a continuous solid

pavement, as on the Blake plateau off the Florida and Georgia coasts. Mero (21) reports a maximal manganese content of 41% on a dry-weight basis, an average of 24.2% and a minimum of 8.2%, for four samples from the Atlantic Ocean. The manganese concentration in sea water depends on the sampling depth (22), with dissolved manganese reaching concentration levels of 5 µg/L at 1000 m depth in the Pacific Ocean, tailing off to 0.4 µg/L at 3500 m. Particulate manganese concentration levels also vary with depth, but not in a systematic manner.

A.2.2 **Anthropogenic**

A.2.2.1 **MMT production** MMT is not manufactured in Canada, but is produced by the Ethyl Corporation in Orangeburg, South Carolina, U.S.A. (3). According to Jaques (4), 866 tonnes of MMT were imported into Canada in 1984, increasing to 906 tonnes in 1985. CMT is available from Strem Chemicals, Newburyport, Mass., U.S.A.

A.2.2.2 **MMT use** MMT, used as a smoke suppressant in fuel and diesel oils (3), is mainly used, in Canada, as an octane booster for both leaded and unleaded gasolines, in which the average concentrations are 3 mg/L and 13 mg/L, respectively (4). It is no longer used in jet fuels (personal communication from Mr. A.E. Spence of the Ethyl Corporation).

A.2.2.3 **Emissions**

A.2.2.3.1 **Industrial emissions of manganese** The anthropogenic sources of manganese in Canada have recently been reviewed by Jaques (4), and much of the material in the following section is derived from this publication, which documents the emissions across Canada for the year 1984.

Canada does not mine manganese ores; known deposits are not presently commercially viable, and, consequently, over 100 000 tonnes of elemental manganese equivalent are imported yearly in the form of ores, concentrates and minerals. The processing of these materials is responsible for the bulk of the manganese emitted into the Canadian atmosphere. Table A.1 summarizes these emissions.

A.2.2.3.2 **Industrial processes** The production of ferromanganese and silicomanganese alloys was responsible for the major portion of the manganese emissions in Canada during 1984 (47% of the total manganese emitted). All production is in Québec. According to Jaques, as a result of increases in alloy production, the emission levels increased by about 56% from 1984 to 1985, such that alloy production is now clearly responsible for more than half of Canada's manganese emissions. The second largest source of emissions stems from primary iron and steel production (27.5% of the total in 1984), two thirds of which is in Ontario. The third largest source of manganese air pollution is a result of the processing of scrap iron and steel (3.3% of the total in 1984). Other industrial processes, such as the production of copper, nickel and zinc, and the manufacture of (manganese) alkaline batteries, cement, welding rods and coke, account for a further 0.7% of the total manganese emission in Canada.

TABLE A.1 Summary of manganese emissions in Canada, 1984
[From Jaques (4)]

Sector	Tonnes	% of Total
Industrial processes	961	78.5
Fuel combustion- stationary sources	20	1.6
Fuel combustion- transportation sources	211	17.2*
Solid waste incineration	5	0.4
Miscellaneous, mainly pesticides	28	2.3
TOTALS	1225	100.0

* MMT-derived manganese emissions used to calculate its portion of total uptake of manganese from all sources in Table E.7.

A.2.2.3.3 **Fuel combustion by stationary sources** Two thirds (67.1%) of the electricity generated in Canada during 1985 was produced by hydro-electric generators. The rest of the electricity (32.9%) was produced by coal-fired stations (18.0%), oil-fired stations (1.6%), stations using natural gas (0.4%), and nuclear power plants (12.9%). In addition to their use for power generation, coal and oil are used for various industrial, commercial and residential purposes. Amongst the fossil fuels, coal is the main source of manganese emissions, with North American coals having a manganese concentration ranging from 3 to 526 ppm, as compared to 0.005-1.45 ppm for U.S. crude oils. From Jaques's data, it can be calculated that coal was responsible for 1.5% of the total anthropogenic manganese emissions during 1984.

A.2.2.3.4 **Emissions due to MMT combustion** Gasoline and diesel oils do not usually contain manganese. The estimated 211 tonnes of manganese emitted by vehicles during 1984 is due to the presence of the octane enhancer MMT, which, on combustion, yields mainly manganese (II, III) oxide (see section A.1.1.3). Neither MMT nor chemically similar octane boosters/smoke suppressants are present in the fuels used by light aircraft (4) or in jet fuels (Ethyl Corp., personal communication from Mr. A.E. Spence); all of the manganese emitted from mobile sources in Canada is due to on- and off-road gasoline-powered vehicle use. The 866 tonnes of MMT imported into Canada during 1984 can maximally give rise to 211 tonnes of manganese equivalent in the form of Mn_3O_4. These 211 tonnes represented 17.2% of the total anthropogenic manganese emissions during 1984 (see Table A.1).

A.2.2.3.5 **Other emission sources** Incineration of municipal waste and sewage sludge, along with the manganese in pesticides (i.e., Maneb and Mancozeb) accounted for a further 2.8% of the total anthropogenic emissions of manganese during 1984.

A.3 **Environmental concentration levels of manganese** The degree of human exposure to manganese varies widely, depending on factors such as the soil manganese concentration levels (food), the vicinity to sources of industrial

pollution and meteorological conditions. This section will attempt to give an overview of the ranges of manganese found in air, water, soil and food.

A.3.1 **Air** Eighty per cent of the manganese present in the atmosphere is associated with particles having a mass median diameter (mmd) of less than about 5 μm. In this section, we are primarily concerned with the concentration level of manganese; but, it should be borne in mind that the distribution of particle size is of the utmost importance for two reasons: firstly, the dispersion of particles from a source of contamination will be determined by size-dependent factors, such as the washout factor and the deposition velocity (23; 24). Secondly, the toxicity of manganese particles inhaled is, also, critically dependent on the particle-size distribution (25; 26).

In a survey of global ambient air manganese concentration levels, the WHO advisory committee concluded that, in non-polluted areas, the levels range from 0.01-0.03 $\mu g/m^3$; in urban-rural areas without significant pollution, annual averages are in the range 0.01-0.07 $\mu g/m^3$, while, in polluted districts, the levels range from 0.2-0.3 $\mu g/m^3$. In industrial areas, where ferro- and silico-manganese alloys are produced, the levels can exceed 0.5 $\mu g/m^3$ (15).

These figures appear to be generally applicable throughout Canada. The most complete data available stem from the Ontario Ministry of the Environment. A complete compilation of trace metal concentration levels in air, for Ontario, during 1982, was also published by Chan et al. (27). For 20 stations, the concentration levels range from 0.0014 $\mu g/m^3$ (in Pickle Lake, Northern Ontario) to 0.0111 $\mu g/m^3$ (in Colchester, Southern Ontario), with a mean concentration (i.e., of the geometric means) of 0.0053 +/- 0.0030 $\mu g/m^3$. These values, thus, fall below the range given by the WHO for non-polluted areas.

Every year, the Ontario Ministry of the Environment publishes data on the ambient air concentration levels of trace metals within the province (28; 29; 30; 31). At the time of completion of this report, data were available up to 1985. The data for the four years 1982-1985 are given in Table A.2 below.

TABLE A.2 **Ambient air concentration levels of manganese in Ontario for the years 1982-1985. ($\mu g/m^3$)***

	1982	1983	1984	1985
Number of stations	58	64	57	56
Geometric mean	0.0411	0.0436	0.0492	0.0599
Standard deviation	0.0560	0.0623	0.0681	0.0742
Range	0.006-0.310	0.001-0.374	0.003-0.344	0.012-0.408

* Calculated from data in references (28; 29; 30; 31).

There was a clear tendency for the ambient air concentration levels to increase throughout these 4 years; the increase can be approximated by the equation:

$$y = 0.0062x + 0.0267 \text{ (in } \mu g/m^3) \qquad (r = 0.958)$$

where y is the concentration level of manganese in the atmosphere in $\mu g/m^3$, and x is equal to: [2 + (z-1982)] and z is the year.

Using this relationship, one can predict the ambient air levels for 1986 to 1988 with a reasonable degree of confidence. The predicted values are given in Table A.3 below.

TABLE A.3 Predicted ambient air concentration levels of manganese in Ontario for the years 1986-1988*

	1986	1987	1988
Levels, $\mu g/m^3$	0.0639	0.0701**	0.0763

* Values calculated from the equation $y = 0.0062 \times (z - 1982 + 2) + 0.0267$ where y is the concentration of manganese in the air expressed in $\mu g/m^3$ and z is the year. The correlation coefficient (r) between air concentration levels and year is 0.958 for the years 1982-1985. This value of r is significant at the 95% confidence level. The equation was calculated from data in Table A.2.

** The predicted 1987 ambient air manganese concentration in Ontario (rounded to 0.70 $\mu g/m^3$) was used to calculate lung intake of manganese in Table E.5. 0.07

It should be noted that, from 1982-1985, manganese levels increased, in contrast to those of other metals (e.g., lead, iron and copper), which were either stable or declined over the same period (31). It should also be noted that the mean values, calculated from the Ontario Ministry of the Environment data for 1982-1985, are significantly higher than those given by Chan et al. (27). This is mainly due to differences in the location and number of stations, the Ministry of the Environment having also sampled air manganese in heavily polluted industrial areas such as Hamilton (n=8) and Sault Ste. Marie (n=3). Chan chose the sites ".. according to a very stringent set of criteria to ensure that they were regionally representative..", and "..illustrate the spatial pattern of precipitation and air quality.."

A.3.2 **Fresh water** Since manganese compounds, commonly found in the earth's crust, are only slightly water soluble, the freshwater manganese concentration levels (both particulate and soluble) are usually less than 50 $\mu g/L$ (3). In natural waters, the solid phases: MnO_2, Mn_2O_3, and Mn_3O_4 appear to be the most important particulates, while $Mn(OH)_2$ may be present at low electrode potentials (i.e., Eh), and $MnCO_3$ may be of consequence in high-salt systems (32). Data from the Canadian "National Water Quality Data Bank" (i.e., NAQUADAT) for the years 1974-1976 indicate that only three areas recorded levels higher than 50 $\mu g/L$; sixty-seven percent of the 84 sampling sites had levels less than 20 $\mu g/L$ (33). In contrast, the concentration levels of

manganese in many areas of Nova Scotia were particularly high, in one case reaching 4.6 mg/L.

There have been a number of studies on lake water concentration of manganese in Canadian and U.S. lake waters, including two undertaken in Ontario, and one in the Adirondack region of the State of New York. In an early study (34), a two year average of 257 µg/L was recorded in Lumsden Lake for 1972 and 1973 as compared with 3 µg/L in the experimental lakes area (in northwestern Ontario near the Ontario-Manitoba border) in September 1973. In a more recent publication (35), Yan and Miller showed that the concentration levels of manganese, in three lakes of the Sudbury basin (Ontario), were elevated, the highest occurring in the lake closest to the Sudbury smelting works. In the Adirondack region, an area known to be sensitive to acidic precipitation, manganese levels, in several lakes and reservoirs, ranged from 4 to 79 µg/L, the mean and standard deviation being 40.5 +/- 27.9 µg/L, respectively (14). Beamish and Van Loon (34) suggested that, irrespective of their location, acidic lakes contain elevated concentration levels of manganese, a relationship which is now generally accepted. The interrelationship between acidity, and the solubility of manganese and aluminum compounds in aqueous solution, has recently been reviewed (36).

These figures, from Ontario and the State of New York, are in good agreement with those found in other geographic locations (15). Tabacova (37), reviewing data that include eastern Europe and Japan, gives the usual concentration level of manganese in drinking water as being 5-25 µg/L, with higher concentration levels usually associated with either contamination of the water by industrial discharges, or with water draining from mineralized areas.

The 1972 concentration level of manganese, in rain-water near Sudbury, Ontario, was about 20 µg/L, whereas less than 1 µg/L was found in snow, believed to be non-polluted, from northern Manitoba (34).

A.3.3 Food The manganese content of various foodstuffs varies over a wide range, and, as a consequence, the dietary intake of manganese is greatly influenced by age, as well as by cultural and socio-economic factors. The highest concentration levels of manganese, on a dry weight basis, are to be found in tea (i.e., 780-930 mg/kg) (38) and in shellfish (i.e., 8-111 mg/kg) (39). However, amongst the various foods which constitute a major proportion of the Canadian diet, the cereals represent the major sources of manganese intake. Kirkpatrick and Coffin (40) divided the Canadian dietary components into 12 groups, and showed that the manganese content, of a representative diet in Vancouver, was highest for cereals, followed by oils and fats, leafy vegetables, root vegetables, legumes and drinks [in decreasing order]. The lowest amounts of manganese were found in milk and other dairy products, and in meat, fish and poultry. Broadly similar results were obtained by Méranger and Smith a few years earlier (41). Kirkpatrick and Coffin's data are summarized in Table A.4. Detailed Tables of manganese concentration levels are to be found in Geigy's handbook of biological data (42), as well as in the U.S. EPA health assessment document for manganese (43), which also contains numerous references to the original analyses.

TABLE A.4 Manganese concentration levels in various food groups of a representative Canadian diet in the Vancouver area, 1970
[From Kirkpatrick and Coffin (40)]

Group number	Constituents	Manganese content*
3	Cereals	9.52
10	Oils and fats	4.37
5	Leafy vegetables	3.68
7	Root vegetables	2.92
6	Legumes	2.23
12	Drinks	2.09
11	Sugars	1.90
9	Fruits	1.86
4	Potatoes	1.77
8	Garden fruits	1.08
2	Meat, fish & poultry	0.33
1	Milk & dairy products	0.12

* mg/kg on a fresh weight basis.

Because of age-dependent changes in the type of food ingested, the dietary intakes of manganese vary widely from the neonate to the adult (see section B.7). The manganese concentration levels in milk from various mammals is lowest in humans (i.e., 7-10 μg/L), and highest in rats (i.e., 140-165 μg/L), with bovines having around 30 μg/L. Infant formula has around 73 μg/L.(44)

Concentration levels of manganese in the Canadian diet have been estimated by Méranger and Smith as being 4.1 mg/diem (41). Other values (see section E.2.1.1) are also given in Table A.5.

TABLE A.5 Daily intakes of manganese in the human diet

Manganese content in daily diet (mg)	Diet studied	Reference
4.7	Canadian*	This study
4.1	Canadian	(41)
3.3	Canadian	(40)
8.3	Indian	(45)
3.8	Japanese	(46)
3.7	International	(47)
2.7	New Zealand	(48)
2.7	U.K.	(49)

* Mean of the three Canadian estimates is 4.03 +/- 0.70 mg/diem.

A.3.4 **Soil** In soil, the manganese concentration levels depend on a number of factors; geological characteristics (i.e., parent rock composition and past weathering processes), and organic matter content, and type of decomposition of the soils are of prime importance (36). The manganese content of the soil is, however, further modified by recent weathering and leaching processes, which, in turn, are pH-dependent. Other factors which can influence the soil manganese concentration levels, are: uptake by the microorganisms, plants and trees found in the local environment, as well as the degree of oxygenation of the soil, the amount of precipitation, and the range of temperatures to which it is exposed.

As mentioned in the previous section, acidic precipitation is believed to affect the concentration levels of manganese in lake waters. In soils, the effect of acidic precipitation and of the soil's natural pH, on the chemical form and bioavailability of manganese, have recently been reviewed (section A.2.1.1; 36). In summary, it can be stated that, in neutral and alkaline soils, manganese exists primarily in an insoluble form, while, at lower pH's, much of the manganese, which occurs primarily in the form of oxides, may become dissolved, thus becoming available for plant uptake.

According to Bohn et al. (50), manganese is usually found at concentration levels ranging from 2-20 µmol/L (110-1100 ppm) in most temperate soil solutions, but concentration levels of 240-750 µmol/L have been found in soil solutions of Typic Hapluduts on Coastal Plains sediments (51). According to Warren et al. (52), the average manganese content of soils in Canada, Great Britain, and certain areas of Wisconsin is 800 ppm, with a deviation of +/- 400 ppm. Wright et al. (53), after studying four major Canadian soil groups, gave a similar range of manganese content: 250-1380 ppm. A report from the U.S. Geological Survey, based on samples from 863 sites throughout the U.S., showed a manganese content ranging from less than 1 ppm to as high as 7000 ppm, with an arithmetic mean of 560 ppm (54).

B BIOLOGICAL FATE OF MANGANESE-CONTAINING PRODUCTS

Many studies of the regulation mechanisms of manganese concentration levels in the human body, and of experimental models, have been reported. The object of these studies has been to gather sufficient information to allow an accurate assessment of the manganese balance. However, at the present time, there are relatively few reliable sets of data on the absorption, retention and body burden of manganese. For example, figures for the percentage of absorption and excretion vary widely, while the total body burden figures of Cotzias (55) are still widely quoted, for lack of better data. A 1972 paper, by McLeod and Robinson (56), summarized the situation at that time, and is still a fair assessment of the present situation: "... except for the studies of Kent and McCance (57).....each study has indicated a daily accumulation of manganese in both normal men and women which approaches in a matter of weeks the estimated total body pool of 12-20 mg manganese....". Although the present view is that manganese is only accumulated to a very small extent in normal adults, reliable experimental studies to support this view are still few and contradictory. In the following sub-sections, an attempt will be made to critically evaluate the data reported in the literature.

B.1 Manganese Absorption

B.1.1 **Gastrointestinal tract** Manganese is taken up by an active transport process in the duodenal and ileal parts of the small intestine, and transported to the liver, from where the bulk of the absorbed metal is subsequently eliminated in the bile. By its very nature, absorption is a dynamic process; following the ingestion of a diet which contains manganese, the amount of the latter which crosses the wall of the gastrointestinal tract (i.e., absorption) will vary with time. Absorption of a given amount of manganese, although initially rapid, slows down with time, such that complete absorption may only occur within a period of tens of hours, depending on the dose and species studied.

There are very few studies in which the percentage of manganese, absorbed across the wall of the gastrointestinal tract, has been measured with a reasonable degree of accuracy. However, there are also data, most often based on balance or elimination studies, which give an estimate of retention. Since not all of the absorbed manganese is retained in the body, the absorption figure will always be greater than that for the retention, and the retention value can be regarded as a minimum value for absorption. Unfortunately, in many papers, there appears to be semantic confusion regarding absorption and retention. As will be discussed in section B.6.3, retention values, given in the literature, range from 1% (60) to 12% (56), all of which are probably too high (see comments in the introduction to section B). The absorption figure (see Table B.1) is considered to be at least 4% for adults, with considerably higher values for young animals and infants.

TABLE B.1 Absorption of manganese from food in the gastrointestinal tracts of mammals

Species	Age	% Absorption	Reference
Man	Infant	99.0	(12)
Man	Young adult	8.4 - 8.9	(61)
Man	Adult	< 3.0	(62)
Man	Adult	1.0 - 4.0	(60)
Man	Adult*	1.7 - 14.5	(63)
Mouse	Adult	0.5 - 2.0	(64)
Mouse	Adult	1.0	(65)
Rat	Weanling	16.0 - 30.0	(44)
Rat	Adult	2.5 - 3.5	(66)
Rat	Adult	1.0 - 2.0	(62)

* The mean value for human adults was calculated as being 5.56% +/- 3.26%; this value (rounded off to 5.5%) is used in Table E.4 to calculate dietary uptake of manganese.

One of the few radioactive tracer studies undertaken in humans was reported by Sandstrom et al. (61). Fourteen healthy subjects (sex unspecified), ranging in age from 20-38 years, were fasted overnight, and then received a test meal containing aliquots of ^{54}Mn and ^{51}Cr. The whole body retention of the manganese was measured daily for the first two weeks, and, subsequently, once a week for up to six weeks. Feces were also collected for the first two weeks. From these data, both the half-times of elimination (see section B.4.1.1) and the absorption of manganese were estimated. The subjects absorbed 8.4% of a test meal consisting of infant formula, and 8.9% of another test meal consisting of a multi-element preparation plus 2.5 mg of manganese sulphate. In a more recent report (63), Sandstrom examined manganese absorption in 14 humans subjects (the same as in his previous paper?), and found a wide variation in the absorption values, which ranged from 1.7%-14.5%. However, the absorption within each subject was highly reproducible.

A number of papers have reported absorption data derived from experimental studies on rats and mice. Amongst the older investigations, Pollack et al. (66) orally administered $^{54}MnCl_2$ to adult rats, and obtained absorption values of about 2.5%, whereas Greenberg (67) arrived at a value of about 4%. For mice, Suzuki (64) reported an intestinal absorption of only 0.5%-2.0% when these were fed very high (i.e., 20-2000 mg/kg) concentration levels of $MnCl_2$. More recently, Van Barneveld and den Hamer (65) examined the apparent absorption of manganese in mice, following parenteral administration of carrier-free ^{54}Mn, parenterally or orally, via food or water. Of the manganese administered in the water, 5.3% was absorbed as compared to only 1.0% absorption from food.

In a study on the absorption of manganese in rats, Garcia-Aranda et al. (58) observed that, in the adult male rat, manganese absorption is a rapidly saturable process, which is independent of pH within the 5.0 - 8.0 range. Data on the kinetics of absorption suggest that absorption in the upper segments of the small intestine occurs more readily than in the ileum.

Data on the absorption, by weanling rats, of ^{54}Mn from labelled milk diets are given by Raghib et al. (44). Eight to thirteen day old rats were given 0.1 mL of labelled milk by stomach intubation, and, subsequently, monitored using a gamma counter at 1-minute intervals. At three hours, more than 90% of the milk had passed through the stomach, and essentially no ^{54}Mn had been lost through the urine or feces. At this time, the animals were killed, and radioactivity in the carcass, digestive tract, and liver were measured separately. In one group of 38 nine-day-old suckling rats who had been fed a variety of milk diets, the absorption was 30.0% +/- 1.7%. The percentage of manganese absorbed fell rapidly with increasing age, the amount absorbed at 13 days being only 16%. This high concentration level of absorption in young mammals is well known (59), and will again be referred to in subsequent sections.

The absorption values, via the gastrointestinal tract, are summarized in Table B.1. Readers are also referred to section B.6.1 concerned with the mechanism of homoeostasis; these refer to data, from rat experiments, which show that the percentage absorption varies with the manganese intake.

B.1.2 **Inhalation** This route of absorption, though of little significance when regarded from the point of view of the daily total intake of

manganese, is of great importance in light of the possible health hazards of manganese-containing particulates, whether from an industrial emission source, or from ambient air particles originating from the combustion of MMT. The amount of manganese inhaled is dependent on a number of factors, of which one of the most important is particle size. Deposition is inversely proportional to particle diameter, and typically reaches 60% for small (i.e., 0.05-0.01 μm) particles. However, in none of the papers dealing with absorption by inhalation, have deposition factors been considered.

As with absorption from the gastrointestinal tract, absorption from the lungs is a dynamic process, and the rate of transfer of the manganese from the lungs to the rest of the body initially proceeds at a fast rate, but concentration levels off to an almost insignificant rate. Similarly, excretion, occurring within the same time-frame, complicates the interpretation of the experimental data; hence, the lung absorption figures should be regarded as approximations. In spite of the clinical importance of this route, there do not appear to have been any quantitative studies on absorption of manganese from the human lung since the work described by Mena et al. (116). These authors found that 40%-70% of the initially deposited radioactive manganese, in the form of $^{54}MnCl_2$ or $^{54}Mn_2O_3$, was excreted within 4 days. These data demonstrated that the absorption and subsequent retention of manganese, via the pulmonary route, was far greater than through the gastrointestinal route.

For animals, numerous experimental studies have been described in the literature. In a study utilizing adult female mice (4-6 weeks old), Adkins et al. (7) exposed the animals, under controlled temperature and humidity conditions in specially constructed chambers, for a two-hour period to Mn_3O_4 aerosols. Particle size analysis of the aerosol showed a mmd of 1.40 +/- 0.09 μm, with 46% of the particles being smaller than 1 μm. The deposition of particles in the lungs, following a 2-hour exposure, was linearly proportional to the manganese concentration. Twenty-four hours after a 2-hour exposure to a 1.80 mg/m^3 aerosol, the manganese content of the lungs was shown to be 14% of that initially deposited. At 48 hours, only 12.4% of the manganese remained in the lungs.

Corresponding studies by Bergstrom (69), who subjected guinea pigs to acute concentration levels of MnO_2 (i.e., 87% of the particles < 3.0 μm; exposure to an aerosol of 22 mg/m^3 for 24 hours), showed that 49.70%, 27.2% and 3.2% of the deposited manganese remained in the lungs at days 1, 2 and 7, respectively.

Drown et al. (16) administered either $^{54}MnCl_2$, or $^{54}Mn_3O_4$, intratracheally to adult male rats, and followed the cumulative excretion of the radioactivity over a two week period. From their data, it can be seen that approximately 75% of the chloride and 65% of the oxide had been excreted by the end of three weeks.

The lung absorption data are summarized in Table B.2; it shows that approximately 70% of the manganese, deposited in the lungs, is subsequently removed and/or taken up in the body. A comparison with the data in Table B.1 shows that absorption from the respiratory tract is an order of magnitude greater than from the gastrointestinal tract.

TABLE B.2 Absorption of particulate manganese inhaled into the adult mammalian lung

Species	Sex	Compound	Aerosol conc. mg/m^3	Absorption %	Ref.
Man	-	Mn_3O_4	-	40 - 70*	(116)
Guinea pig	-	MnO_2	22.0	70 - 80	(69)
Mouse	F	Mn_3O_4	1.8	88	(7)
Rat	M	Mn_3O_4	0.055	65	(16)

* The maximum absorption (70%) of inhaled particulate manganese is used to calculate uptake of manganese from the lung per day.

B.2 Distribution of manganese

B.2.1 **Transport mechanisms** From the gut lumen, a relatively small fraction of the manganese is taken up in the mucosal cell, and is subsequently transported in the blood stream to the liver and other organs. In the liver, the manganese is transferred to the bile, and subsequently excreted.

According to workers in Valberg's group (70; 71; 72), manganese, iron and cobalt compete for uptake into the mucosal cell, perhaps through competition for the same receptor. Manganese is postulated to be taken across the mucosa by a high affinity, low capacity, active transport mechanism. Others have suggested that intestinal absorption of some trace elements, including manganese, may be linked to low molecular weight substances, which form complexes with the metal, thus facilitating its transport across the mucosa. Thus, in a study by Garcia-Aranda et al. (58), absorption rates of manganese, as studied by perfusion experiments in the rat, were enhanced 3-4 times by histidine and citrate.

Manganese absorption takes place in two distinct steps, the manganese first being taken up from the gut lumen into the mucosal cell, followed by its transfer to the blood circulation system (71). Once in the blood stream, manganese binds to an 80 KD protein, which is believed to be transferrin (73), and is taken up in the liver. Some workers support the view that transferrin is only one of several plasma manganese-transporting compounds (74). In the liver of mice, the manganese is rapidly taken up by both mitochondria and lysosomes, and is subsequently excreted in a low molecular weight form, which can co-migrate with manganese acetate on Sephadex G-10 columns (75). This manganese-bearing compound is then released from the lysosomes of the hepatocytes, and transferred to the bile for subsequent excretion.

B.2.2 **Tissue distribution** The early studies of Cotzias (55), showing that the concentration levels of manganese in mitochondria are particularly high, are widely cited in the literature (e.g., 76). However, work by Suzuki and Wada (75) has brought attention to the fact that, in mice hepatocytes, the lysosomes both take up and excrete manganese more rapidly than do the mitochondria, and that, as a consequence and in spite of their smaller

volume (i.e., 10% of that of mitochondria), the flux of manganese through these organelles is greater than in mitochondria. According to these authors, the maximum concentration levels of manganese in all organelles occur at between 30 minutes and 2 hours after intraperitoneal injection of manganese acetate at a concentration level of 49 mg/kg. By 6-8 hours, these concentration levels decline to 25% of their maximum values, reaching about 0.1 μg/mg protein in the mitochondria and half this concentration level in the microsomes. In contrast, a recent study by Papavasiliou and Miller (77) on the subcellular distribution of ^{54}Mn in the liver and brain of mice (5 minutes after injection of carrier-free $^{54}Mn^{2+}$) showed that, in both the liver and the brain, the microsomes had taken up as much manganese as the mitochondria; subcellular distribution of the metal varies widely in the various regions of the brain, such that, whereas cerebellar mitochondria contain 43.2% of the cell manganese, the cortical mitochondria have only 18.8% of the cell manganese as compared to 37.6% in the microsomes.

The amounts of manganese in various tissues have been compiled by the task group on Reference Man (47). Some of these data, together with the corresponding wet tissue concentration levels, are given in Table B.3., from where it can be seen that, on a weight basis, the main pools of manganese reside in the liver and skeletal muscles, followed by connective and adipose tissues, intestine, and brain.

TABLE B.3 Tissue distribution of manganese in Reference Man
[From Snyder (47)]

Tissue	Weight(g)	Amount of manganese (μg)	Tissue concentration (μg/g)
Total body	70 000	12 000	0.171
Total soft tissues	60 000	7 200	0.120
Skeleton	10 000	5 200	0.520
Liver	1 800	2 500	1.389
Skeletal muscle	28 000	1 500	0.054
Connective tissue	3 400	680	0.200
Intestine	1 000	570	0.570
Adipose tissue	15 000	500	0.033
Brain	1 400	390	0.279
Kidneys	310	280	0.903
Whole blood	5 500	140	0.025
Lung	1 000	120	0.120
Pancreas	100	110	1.100
Heart	330	66	0.200
Stomach	150	46	0.307
Testes	1	4.5	4.500
Thyroid	20	4.0	0.200
Gall bladder	10	2.7	0.270
Adrenals	14	2.4	0.171
Thymus	20	1.8	0.090

However, the testes, followed by the liver, pancreas and kidneys, all of which have 1 μg/g or more of manganese, are the tissues which show the greatest concentration of the metal. The high concentration levels in the liver are partly due to the fact that mitochondria make up almost 20% of the cell volume (76), while the endoplasmic reticulum is also extremely well developed. The values given in Table B.3 are supposedly characteristic for the organ/tissue, across a wide range of mammalian species (78); but, in fact, quite considerable variation can occur. Thus, for liver, the concentrations in the human, rabbit and rat are 1.4 μg/g, 2.1 μg/g and 4.2 μg/g, respectively (47; 79; 80).

Pigmented (i.e., ectodermal) tissues are rich in manganese; this has led some researchers to suggest that the analysis of washed hair be used as a means of estimating environmental exposure (81). The manganese concentrations, in the hair of manganese smelter workers, were greater by factors of more than ten, over those of a control group (i.e., 19.0 μg/g vs.1.2 μg/g).

The manganese content of whole human blood is probably less than 2 μg/100 mL. Values obtained in the 1960's are tabulated in a WHO report (15), and the mean of six estimates of the mean is 3.6 +/- 2.5 μg/100 mL. These values are considerably higher than more recent values. Snyder et al. (47) give a value of 2.55 μg/100 g of blood for Reference Man, which is equivalent to a concentration of 2.41 μg/100 mL; Dupont and Tanaka (82) give an average value of 1.44 μg/100 mL (range 0.8-2.1 μg/100 mL) for 29 children in the age group 2-17 years. Even lower values are given by Roels et al. (83), who quote the range 0.04-1.31 μg/100 mL for 104 normal Belgian adults. This steady decrease in the reported manganese concentration levels is undoubtedly due to refinements in analytical techniques, especially in the elimination of errors in the blanks.

There is, as there can be seen from the ranges quoted above, a considerable individual variation in blood manganese concentration levels. However, several groups of workers have reported that the levels in both the human (83; 84) and the rat (125) are, on a group basis, a reflection of the body burden of manganese. Recent studies by Dupont and Tanaka (82) have also indicated that children with convulsive disorders have significantly lower blood manganese concentration levels (i.e., 0.94 μg/100 mL as opposed to 1.42 μg/100 mL in the control group; both are means of the summer and winter concentration levels).

B.2.3 **Total body burden** Extremely few estimates have been made for the total amount of manganese in the adult human body. The generally accepted values lie in the range of 12-20 mg (85), with the International Commission on Radiological Protection (ICRP) value (shown in Table B.3) being 12 mg.

B.3 **Metabolic and regulatory role of manganese** Manganese is an essential trace element which is known to participate as a coenzyme or as a prosthetic group in a number of enzymatic reactions, which are primarily those involved in carbohydrate metabolism and its regulation. Manganese is also required for the activity of many other enzymes, although the mechanism of action is not known. In addition, manganese has physiological effects which, likewise, are not fully understood, such as its ability to increase blood glucose through stimulating gluconeogenesis (86; 87), and to abolish the insulin-releasing action of glucose (88). Besides the specific processes given in Table

B.4, manganese is also said to be involved in the non-specific activation of a number of enzymes such as hydrolases, kinases, decarboxylases and transferases (89; 90), as well as in the regulation of thiol-esterases and adenyl cyclase (90; 91).

TABLE B.4 **Known biological roles of manganese**

Enzyme	Involvement of manganese	Process	Ref
Pyruvate kinase	Activates	PEP->P	(92)
Pyruvate carboxylase	Metalloenzyme	P-->OA	(93)
PEP carboxykinase	Metalloenzyme	OA-->PEP	(93)
Superoxide dismutase	Metalloenzyme	Radical scavenge	(94)
Tyrosine hydroxylase	Regulates	Tyr->DOPA	(95)
Prolidase	Required	Gly-Pro cleavage	(78)
Succinic dehydrogenase	Required	Succ-->Fum	(78)
Arginase	Required	Arg->urea + ornith	(96)
Glutamine synthetase	Metalloenzyme	Glu->Gln	(97)
Glycosyl transferase	Required	Carbohyd.Synth.	(98)

Arg Arginine; Fum Fumarate; Gln Glutamine; Glu Glutamic acid; Gly Glycine; OA Oxaloacetate; Ornith Ornithine; P Pyruvate; PEP Phosphoenolpyruvate; Pro Proline; Succ Succinate; Tyr Tyrosine

B.4 Metabolism of manganese

B.4.1 Biological half-times

Following parenteral administration, the elimination of manganese is biphasic, with an initial rapid phase, corresponding to the rapid removal of manganese from the circulation, chiefly by the liver, and its subsequent excretion after discharge of the bile into the gut. This is followed by a slower rectilinear phase on the retention curve, which corresponds to the steady turnover and subsequent excretion of manganese from various equilibrated body pools. Furthermore, following oral administration, an initial and even more rapid phase is seen, which reflects the excretion of that fraction of the manganese which has never been bound to the mucosal cells, but simply passes through the gut. This phase overlaps the rapid excretory phase corresponding to removal of manganese from the blood circulation system by the liver, and it may be difficult to deconvolute the differing half-times of the two processes from the retention curve.

B.4.1.1 **Humans** For humans, very few experimental data exist. The work of Mahoney and Small (99), involving six volunteers, showed that whole-body elimination of an injected dose of ^{54}Mn could be described as a two-phase process with half-times of 4 and 39 days, respectively. They also showed that the rates of elimination were dependent on the amount of manganese

already present in the body as a result of previous oral administration. In the same year, Cotzias et al. (100) described whole body clearance in terms of a single half-time of 37.5 days, and was one of the first to demonstrate that elimination of manganese from various parts of the body occurred with widely differing kinetics. Thus, manganese was cleared from the blood stream with a half-time of 1.5 minutes, while the half-times of clearance for the liver, head and thigh regions were 25d, 54d and 57d respectively. More recent work, by Sandstrom et al. (61), showed that orally administered ^{54}Mn was eliminated with half-times of 13 and 34 days (n=14); ranges for the half-times were 6-30 and 26-54 days, respectively. In two other subjects, the half-times were 8 and 15 days following oral administration, but, when manganese was administered intravenously 6 months after the oral dose, the half-times were 23 and 65 days, demonstrating a much slower turnover following parenteral administration. About 40% of the ^{54}Mn activity was located in the liver, which showed a similar turn-over rate to that of the whole body.

B.4.1.2 Animal data Numerous experiments, often of a more qualitative nature, have been carried out using animals, especially rodents. Dastur (101) gave the half-time for whole-body elimination of manganese from monkeys as being 95 days, and noted that the brain concentration levels were still high after 9 months.

Many studies have been published concerning the manganese elimination in rats, but, unfortunately, very few have reported kinetic data. The study of Garcia-Aranda et al. (58) is of particular interest, since it is one of the few to describe the kinetics of absorption of manganese from the gut lumen into the jejunal wall. In their experiments, 125 µmol $MnSO_4$ were perfused in the absence of other chemicals, and the absorption rate was found to be 16 pmol/min/cm^2, 30 minutes after beginning the sample collection, declining to 2.3 pmol/min/cm^2 at 90 minutes. The estimated initial rate of absorption was 66 pmol/min/cm^2.

Though no half-lives are given, Drown et al. (16), in their study of the clearance of $^{54}MnCl_2$ and $^{54}Mn_3O_4$ from rat lungs, showed that the initial clearance of the chloride was approximately four times faster than that of the oxide (with half-times of about 1 and 4 days, respectively), but that, after 2 weeks, the amount remaining in the lungs was similar. Fifty per cent of the dose of $MnCl_2$ was excreted during the first 3 days, whereas it took one week to excrete 50% of the oxide (by whole-body kinetics).

A recent study by Kalliomaki et al. (102) gives kinetic data on the clearance of welding fume manganese from the rat lung. The half-times differed according to the fume type. For manual metal arc/mild steel (MMA/MS) fumes, the half-times were in the same range as those given by Drown (16). The fast half-time was 0.53 days and the slow half-time was 4.3 days, with only 10%-20% of the manganese being removed through the fast kinetics. However, when welding stainless steel by either the MMA or the metal inert gas (MIG) technique, the overall half-times were an order of magnitude higher. For MMA/SS and MIG/SS, the respective values were 5/40 days and, approximately, 107 days respectively, with about 80% and 100% being removed by the slow process in each case. For MIG/SS, the lung clearance for chromium and iron was even slower with half-times being greater than 240 days.

In mice, Adkins et al. (7) studied the lung retention of Mn_3O_4 following a two hour exposure period during which an aerosol, containing particles of mmd 1.4 μm was inhaled. From their data, it can be calculated that the half-time of lung retention was 3.6 hours, with about 12% of the deposited material still retained after 24 hours. This half-time value is remarkably short, when compared to other estimates of lung clearance (see Table B.5). The paper by Adkins is one of the few in which the amount of particulate, deposited in the lung after exposure, was measured.

TABLE B.5 Clearance of manganese from mammals

Species	Organ	Compound & Route		Half-times of clearance Fast	Overall	Slow	Ref.
Human	Lung	MnO_2	i	16h		50d	(103)
Human	W.B.	^{54}Mn	o	4d		39d	(99)
Human	W.B.	^{54}Mn	o	13+/-8d		34+/-8d	(61)
Monkey	W.B.	^{54}Mn			95d		(101)
12d rat	Intestine	Mn_3O_4	o	2.5-5.6		13.7-21.5	(59)
24d rat	Intestine	Mn_3O_4	o	2.0-7.5			(59)
Rat	Lung	Welding fume	i	0.53/5d	107d	4.3/40d	(102)
Rat	Lung	$^{54}MnCl_2$			ca.1d		(16)
Rat	Lung	$^{54}Mn_3O_4$			ca.4d		(16)
Rat	W.B.	$^{54}MnCl_2/^{54}Mn_3O_4$			ca.3d/7d		(16)
Mouse	Lung	Mn_3O_4			3.5h		(7)
Mouse	W.B.	^{54}Mn	o/iv		1-6d*		(64)
Mouse	W.B.	^{54}Mn	o/ip	<1d		8.4d	(65)
Mouse	W.B.	$^{54}MnCl_2$	o	2.5d		19d	(104)

W.B. Whole body iv intravenously o oral
i inhalation ip intraperitoneally
d days h hours {depending on oral dosing (20 - 2000 mg/L) prior to iv of ^{54}Mn}

* Depending on oral dosing (20 - 2000 mg/L) prior to iv of ^{54}Mn.

Suzuki (64) studied the whole-body clearance of ^{54}Mn in mice, the latter having previously received manganese chloride in high concentrations (i.e., 20-2000 μg/L in water) for 26-30 days prior to the clearance experiments. The half-times varied from 6 days for the 20 mg/L group to 1.0 -1.5 days for the 2000 mg/L group, confirming the observations, made by Mahoney and Small (99), indicating that clearance in the adult human is more rapid following previous exposure to a higher manganese load.

Table B.5 summarizes the clearance data discussed above, as well as other studies.. The following conclusions can be drawn:

- overall clearance estimates tend to be closer to the rapid initial clearance values (e.g., comparison between the 4 day estimate for overall turnover from Drown's data on rat lung clearance with the 4 day/18 day values given by Rehnberg).

- whole body half-times of elimination range from 1.5/13.5 days for mice, and an overall value of 3-7 days for rats versus the 8.5/36.5 days for humans. The overall value of 95 days for monkeys, given by Dastur, stands out because of its duration.

- values for lung clearance vary from a half-time of 3.5 hours for mice (Adkins) to 60 days (Morrow) for humans. There is a very obvious need for further experimental work to obtain reliable kinetic data on lung clearance.

- the clearance of toxic welding fumes in the rat takes substantially longer than for the other aerosols studied (i.e., by one to two orders of magnitude). The particle size distribution in the fume was, unfortunately, not given, but it can be presumed that the particle size extends from 1.0 μm down to 0.001 μm (105).

- clearance from other organs is highly variable, but, generally speaking, while elimination of manganese from the heart muscle, the brain, and the skeleton is slow, that from the intestine is fast, and clearance from the skeletal muscles and kidneys is intermediate (16).

B.5 Elimination of manganese from the body

B.5.1 **Routes and amounts excreted** Elimination of manganese via the feces is the main excretory route for manganese, apart from early post-natal life, when bile excretion is low (82). A small fraction of the manganese becomes bound to mucosal cells, whereas the rest passes through the gut. Of the manganese taken up in the mucosal cells, an unknown fraction is absorbed, while the rest is lost again, by a process of desquamation of mucosal cells, into the gut lumen. Since the turnover rate of intestinal epithelial cells is 4-5 times slower in the suckling than in the weanling or adult rat (106), the fraction of manganese lost in the feces will be smaller in the neonate. After absorption and transport to the liver (see section B.2.1), the manganese is complexed, transferred to the gall bladder, passes down the bile duct, and enters the gastrointestinal tract.

Small amounts of manganese are also lost in the sweat and the urine. Urinary excretion varies over a wide range. In one study of manganese elimination in humans (107), it was estimated that, with a daily intake of 2273 μg manganese, 13.8 μg/diem were lost in the urine as compared to 2106 μg lost daily in the feces. The urinary excretion, thus, represents 0.65% of the total manganese eliminated daily. Generally, less than 1% of the total manganese excreted is lost via the urine, and Schlage et al. (108) quote a range of from 0.1% to 2%. However, the amount excreted varies with intake, and Snyder (47) quotes values corresponding to 3.6% of the intake for diets containing 3.3 mg of manganese. Whereas the blood manganese concentration level appears to reflect the body burden, it appears that the urinary concentration level reflects recent exposure. In contrast to the low urinary excretion of inorganic ingested manganese, the bulk of orally administered MMT is excreted via the urine in rats

(109), with up to 81% of the compound being eliminated over the first two days, as compared to 2%-3% in the feces.

Sweat contains about 60 μg Mn/L (110), and, assuming a daily sweat secretion of 650 mL (47), it can be estimated that loss of manganese through the skin is about 39 μg. The ICRP values for manganese loss through sweat fall in the range of 20.8-48.1 μg/diem (mean value 35.5 μg/diem). A minor amount of manganese is also lost through sloughing off of the skin, and through menstruation, as well as by the cutting of nails and hair.

B.5.2 **Mechanism** Details of the mechanisms, whereby manganese is transferred from liver cells to the bile, are unknown. As there is a 100-200 fold difference in the concentration of manganese between plasma and bile, there must be considerable expenditure of energy through an active transport mechanism.

Weigand (111) quotes a number of sources, which indicate that the biliary concentration of manganese is regulated in response to dietary changes, increasing rapidly following intraduodenal dosage or intravenous injection. The manganese contained in the bile is believed to be in a different, non-ionic form than that in the diet. In this form, perhaps joined to bilirubin, it is more easily taken up into the enterohepatic circulation system, (see also section B.2.1). Cirkt (112) showed that only 15% of a $MnCl_2$ dose, introduced into the duodenum of a rat, was absorbed, in contrast to some 35% of an equivalent dose of manganese added to the bile.

B.6 **Homoeostasis of manganese**

B.6.1 **Mechanisms** As a result of homoeostatic regulation at the concentration level of both absorption and excretion, manganese does not accumulate in the adult human body (76). A rigorous proof of this would require a study showing how the total body burden varies with age - such studies have never been undertaken, although limited data suggest that the concentration levels in certain tissues are constant (see references in 111). The retention figures, which appear in the literature, indicate that the body burden increases with age, albeit slowly.

Although it was previously believed that homoeostasis involved only the excretory mechanism (15; 43), it is now recognized that there must also be regulation of absorption; the evidence for this can be summarized as follows:

- When rats are fed various concentration levels of manganese in their diet for 2 weeks prior to their receiving an oral dose of $^{54}MnCl_2$, the amount of radioactivity in the tissues varies inversely with the concentration level of manganese in the previous diet (113). If the chief homoeostatic control were at the concentration level of excretion, the tissue concentration levels would have been expected to vary directly with the dietary concentration levels.

- Absorption of manganese in rats, far from being a constant percentage of the dietary intake, decreases with increasing dietary concentration levels (111).

- If there were no homoeostatic control at the concentration level of absorption, tissues could be temporarily subjected to toxic concentration levels of the metal following high manganese intakes. Acute toxicity to manganese, in rats, is known to occur at much higher doses after intraperitoneal, as compared to oral administration (114).

B.6.2 Dietary influence Studies of the absorption of manganese have shown that a variety of organic and inorganic substances have a considerable influence on the amount of metal taken up from the gut. Regarding organic substances, the effect of histidine and citrate has already been referred to (see section B.2.1). Other substances, such as phytate (115), have also been shown to reduce absorption of manganese in the rat, but the interpretation of this effect is complicated by the fact that phytate is able to form a complex with calcium. Calcium is known to suppress the absorption of manganese (see below). Phytic acid is found in a variety of starchy foods (e.g., corn and potatoes) forming complexes with magnesium and calcium producing phytin, but, in the free acid form, it can form complexes with a variety of heavy metals. The effects of a number of inorganic ions on the dietary uptake of manganese have been well documented, and include, in particular, calcium, magnesium, iron, and other transition metals. Most of this work has been done under experimental situations.

Magnesium As discussed in (76) and section B.4.1, manganese and magnesium are chemically similar, such that, in mice, manganese absorption is affected by the dietary concentration level of magnesium. Van Barneveld (65) showed that both calcium and magnesium could reduce the absorption of manganese present in either food or water, and suggested that there might be a competition between these elements both at the concentration level of receptor-binding on the mucosal cell, and during subsequent transport.

Iron Studies in humans have shown that manganese absorption is increased from the normal concentration level of 3% to 7.5%, as observed in anemic miners (116). Furthermore, it was shown that the absorption of manganese correlated with that of iron, with anemic subjects showing a far greater absorption of manganese. This interrelationship between iron and manganese has been supported by animal studies in adult rats; these show that iron-deficiency results in increased manganese uptake and resultant toxicity (71; 117). However, the effect of iron supplementation on manganese absorption is, to a great extent, age dependent. Gruden (118), working with neonatal and weanling rats, showed that iron supplementation in neonates results in a decrease in the amount of manganese retained in various organs. In contrast, iron-supplementation of the milk, fed to weanling rats, at a concentration level of 50-200 μg/mL milk caused an increased retention of manganese. However, increasing the iron supplement to a concentration level of 410 μg/mL, or more, resulted in decreased manganese retention. Gruden interprets these results as follows: in the neonate, the homoeostatic regulation of iron is not yet developed; and, as a consequence, the manganese transport mechanisms become saturated with iron. In the weanling rat, the homoeostatic regulation of iron has developed, such that the body can cope with moderate iron doses, but, at too high a concentration level of iron, the iron transport mechanism becomes saturated, and competition for the manganese transport mechanism occurs between iron and manganese. However, it should be noted that previous work by

Kostial et al. (119) failed to show a decrease in manganese retention in sucklings whose milk had been supplemented with iron. This discrepancy is probably due to the fact that iron was only used as a supplement at one dose concentration level (i.e., 100 μg/mL), at which Gruden found that there was relatively little effect.

Calcium Studies by Spencer et al. (107) of the metabolic balances in humans of a number of metals, including manganese, showed that a calcium intake in the range of 200 to 1500 mg/diem had a small effect on manganese excretion and retention. In the presence of a calcium supplement, the fecal excretion of manganese increased from a mean of 93% to 108% of the dietary intake, resulting in a negative balance (see section B.7). The above results are in concurrence with the experiments on mice mentioned previously, in which high concentration levels of calcium reduced the absorption of manganese from food (65). Calcium depletion, in contrast to magnesium, causes a significant increase in manganese absorption. In support of these results, which suggest an interaction between calcium and magnesium, Chornock (120) showed that rats with a high dietary manganese content developed negative calcium and phosphorus balances, and eventually became rachitic.

Other ions Chandra et al. (121) have shown that animals, co-exposed to manganese and lead, accumulate greater amounts of lead. This metabolic interrelationship has also been demonstrated by studies involving humans who were co-exposed to manganese and lead pollution; in the latter, there proved to be an inverse relationship between the lead concentration in the blood, and the amount of manganese excreted in the urine (122). Competition also exists between manganese, iron, and cobalt for both uptake into mucosal cells, and for subsequent transport (70; 72). All of these interrelationships are summarized in Table B.6.

TABLE B.6 Interrelationship of metal ions with manganese metabolism

Experimental strategy	Effect	Species	Ref.
Decrease dietary Ca	A↑(Mn)*	Rat	(65)
Increase dietary Ca	A↓(Mn)	Rat	(65)
High dietary Mn	A↓(Ca)	Rat	(120)
Increase dietary Mg	A↓(Mn)	Rat	(65)
Decrease dietary Fe (anemia)	A/R↑(Mn)**	Human	(116)
Increase dietary Fe	R↓(Mn)	Neonate rat	(118)
Increase dietary Fe (50-200 ppm)	R↑(Mn)	Weanling rat	(118)
Increase dietary Fe (>410 ppm)	R↓(Mn)	Weanling rat	(118)
Coexposure to Pb and Mn	R↑(Pb)	Rat	(123)
Coexposure to Pb and Mn	E↑(Mn)***	Human	(122)
Increase Fe or Co	R↓(Mn)	Rats, mice	(70; 72)

* A = absorption
** R = retention
*** E = urinary excretion
Increase = ↑
Decrease = ↓

In conclusion, because of the influence of the organic and inorganic components present in the diet, it is not possible to accurately predict, from an elemental analysis, the amount of manganese, present in a diet, that will be absorbed.

B.6.3 **Age dependent changes** In the adult mammal, three separate mechanisms prevent manganese from reaching toxic concentration levels within the body tissues. These are: the intestinal and blood-brain barriers, and the excretion mechanism of the liver. Throughout the period going from birth to weaning, these homoeostatic systems are apparently not fully developed, such that the young will be unable to deal with moderate manganese loads.

Prior to birth, manganese passes the placental barrier in humans and other species, and accumulates in the fetus, such that the new-born typically has tissue concentration levels which are 7%-9% higher than those of adults. After birth, manganese is passed to the child in breast milk (37), but, as discussed previously, the manganese content of human milk is very low (see section A.3.3), such that these elevated tissue-manganese concentration levels are only sustained in the infant for a few weeks after birth. Six-week-old infants still have higher tissue-manganese concentration levels than older children (124). It has recently been shown that the manganese content of whole blood is a valid indicator of the body burden of manganese (83; 84; 125). It is, therefore, not surprising that the newborn has blood manganese concentration levels of about 1.95 μg/100 mL, as compared to 1.55 μg/100 mL and 1.40 μg/100 mL at the ages of 1 and 2 years, respectively (82). Chan et al. (126) report a concentration level of 1.84 μg/100 mL for 2-3 day old full-term infants. That the manganese tissue concentration levels do not fall more rapidly in infants is, presumably, due to both increased absorption from the diet (see section B.1.1) and to diminished excretion (127), but these suppositions are primarily based on experiments with neonatal rats.

Gastrointestinal absorption in the young mammal is markedly higher than in the adult. Mena (68) states that intestinal manganese absorption in the young rat is around 70%, as compared to 1%-2% in the adult, whereas Raghib et al. (44) have confirmed that manganese absorption in young suckling rats decreases rapidly with age. Clarke and Hardy (62) observed that, on the 18th day of life, the young rat abruptly loses the ability to take up the polymer PVP (mean M. Wt. of 160 kilodaltons), and suggested that this is due to repopulation of the intestinal villi with "non-permeable" cells, as a result of some hormonal stimulus on the 18th day. Rehnberg et al. (59) showed that the stomach retention time in the pre-weanling rat was more than twice that of the weanling rat, and that movement of material through the ileum was significantly slower. All of the above factors would increase the potential for absorption of manganese in the neonatal gastrointestinal tract.

Neonatal rats and mice do not excrete manganese for the first 2-3 weeks of life, presumably due to an immature liver (128). Little or no stool is passed, and, therefore, excretion is minimal. The neonatal mammal is, thus, geared to conserve the relatively high tissue manganese concentration levels acquired during fetal life, but ill-equipped, by the nature of its undeveloped homoeostatic mechanisms, to deal with manganese loads. Maljkovic and Kostial (114) showed that manganese toxicity, indicated by LD-50 values, was highest in sucklings (2 weeks old) and in older rats (52 weeks). Mena (68) showed that manganese can

penetrate the blood-brain barrier of young rats at a rate, which was four times that occurring in adults. Others (129; 130) have shown that there is a greater accumulation of manganese in the brain of young rats as compared to adults. Similarly, studies on rats and mice have shown that, when nursing mothers are given high doses of manganese, the neonates accumulate manganese to higher concentration levels than in controls, especially in brain tissue (128).

In accordance with the recognition that the neonate has poorly developed homoeostatic mechanisms, Maljkovic and Kostial (114) found that the LD-50 values for $MnCl_2$ were age-dependent. The highest LD_{50} values were found for 3-6 week old rats, indicating the lowest toxicity for manganese in this age group. Lower LD_{50} values were found in the youngest and oldest age groups (see Table B.7) and, as expected, intraperitoneal administration resulted in much higher toxicity. The higher toxicity in the older animals was attributed to degenerative changes in the kidney. The influence of age on manganese toxicity under occupational settings is discussed in section D.1.

TABLE B.7 Toxicity of manganese chloride in rats of different ages
[From Maljkovic & Kostial (114)]

	LD - 50 values (mg/kg)			
	I.p. route		Oral route	
Age (weeks)	MALES	FEMALES	MALES	FEMALES
2	86*		802*	
3	141	129	1890	1860
6	134	108	1730	1712
20	60	73	962	850
52	61	73	763	619

* Male and Female sucklings

B.7 **Balance studies - requirement and retention of manganese**

Although considerable experimental data exist on manganese balance and retention in the human (see reference in 108), very few of the published balance studies are of value, either because of deficiencies in the analytical methods, or because the study was too short. In a review of balance studies (see Table B.8), McLeod and Robinson (56) showed that short-term studies (i.e., 6-9 days) give retention values of around 29% over a wide range (i.e., 5.6 mg - 22.0 mg) of daily manganese intakes.

McLeod and Robinson, themselves, arrived at a retention figure of 11.5% from an 18 day study of 4 young women. This figure is much higher than those given for Reference Man (47; Table B.8), or for adult males (107). Spencer conducted manganese balance studies on adult males at various calcium intake concentration levels, and found that, at low calcium intakes (i.e., 200 mg/diem), the retention was about 6.7%, whereas, at high calcium intakes (i.e., 200-1500 mg/diem), a negative balance was seen, the mean retention for 13 determinations being -6.7%.

TABLE B.8 **Manganese balance in adult humans**

Intake (mg)	Losses (mg)		Retention (mg) [%]	Ref.
2.78	Urine	0.01	0.32 [11.5]	(56)
	Feces	2.45		
3.702	Urine	0.03	0.031 [0.8]	(47)
	Feces	3.60		
	Sweat	0.039		
	Hair, nails etc	0.002		
2.273 (low Ca)	Urine	0.014	0.152 [6.7]	(107)
	Feces	2.106		
2.165 (high Ca)	Urine*	0.012	-0.178 [-8.2]	(107)
	Feces	2.330		

* The average urinary excretion loss is 0.58% of intake for the four studies reported in this Table.

The retention value of about 1%, given by Snyder for Reference Man (47), is a far more reasonable estimate than the 11.5% value calculated from McLeod and Robinson's data (56). Simple considerations demonstrate that it is highly unlikely that the true retention figure for an adult human can exceed more than a few tens of micrograms. Schlage and Wortberg (108) have tabulated manganese intake data obtained from the literature (see Table B.9), from which it can be seen that, from the time that a child has been weaned, the manganese intake (expressed as mg Mn/kg body weight/diem) is fairly constant, the mean and standard deviation being 0.086 +/- 0.023 mg/kg/diem (for n = 8), respectively.

TABLE B.9 **Daily intake of manganese in humans from birth to maturity**
[Adapted from Schlage & Wortburg, (108)]

Age (years)	Intake* mg/diem	Yearly intake mg/kg/diem	(mg)	Total intake over age range (mg)
0 - 1	0.04	0.009	15	16
1 - 2	0.8	0.08	290	290
2 - 3	(3.2)	(0.27)	(1165)	(1165)
3 - 5	1.4	0.08	510	1020
5 - 6	2.8	0.07	1020	1020
6 - 10	3.8	0.13	1340	5530
10 - 13	2.2	0.06	800	2400
13 - 16	3.2	0.01	1160	3500
16 - 22	4.2	0.09	1530	9170
Adult	4.3	0.07	1550	----

* The same values are found in Table E.1 below.

Assuming that the body manganese burden of a 5 year old child is 6.5 mg, and that that of a 22 year old is 20 mg, 13.5 mg of manganese (i.e., 20-6.5) need to have been accumulated over a 17 year period, from which a daily retention of 2.2 μg/diem (i.e., (13.5 x 1000)/(17 x 365.25)) can be calculated. This retention figure should be compared with the upper limit intake of 6.02 mg/diem (i.e., 0.086 x 70) for a 70 kg adult. The retention must clearly be well under 0.1%. Conversely, were the daily retention to be 10% (i.e., 0.60 mg/diem), the assumed total body burden of 20 mg in an adult would be doubled within five weeks. Further support for a low retention figure comes from animal studies, (104) which show that, in mice, the retention of $^{54}MnCl_2$ was 2.7% after 10 days, and was still decreasing at that time.

Manganese balance has also been examined in the new-born child in attempts to evaluate the manganese requirement of the neonate, whose manganese intake from milk is noticeably lower than in the adult (see section B.6.3). An early report, by Widdowson (131), indicated a strongly negative balance at 1 week. A more recent study, by Zlotkin and Buchanan at the Sick Children's Hospital in Toronto (12), showed positive balance in 24 pre-term and full-term infants receiving manganese, via intravenous infusion, at a rate of 48 μg/kg/diem. The retention was 99%, and was directly proportional to the manganese intake over the range of 0.2 to 70 μg Mn/kg/diem. These authors quote Casey and Robinson (132) as saying that, in the last trimester of pregnancy, a 1 kg infant accumulates manganese at a rate of 9 μg/kg/diem.

Quantitative balance data are not as numerous as intake data; both are summarized in Tables B.8 (balance) and B.9. (intake). Recent studies by Shiraishi et al. (46) are in agreement with these values, giving intakes of 3.53 and 4.02 mg/diem for the inhabitants of Sapporo and Kyoto, respectively.

The manganese requirements of humans have not yet been firmly established (107), but, as deficiency symptoms have never been observed with normal dietary intakes, it can be assumed that a few mg/diem are more than sufficient to maintain good health.

B.8 Manganese deficiency While the clinical expression of manganese deficiency in experimental animals has been described in detail, only one case of deficiency has been reported in humans (133). During a metabolic study, manganese was accidentally omitted from the diet, resulting in weight loss, dermatitis, hair changes, gastrointestinal symptoms and sterility. All of the above symptoms were alleviated on adding manganese to the diet. Generally, deficiency in humans is believed to be rare because of the abundance of manganese in the diet (see B.7).

Hurley et al. (134) showed that the offspring of pregnant rats, who were maintained on a manganese-deficient diet, had defects in cartilage function, and were more susceptible to electroshock induced convulsions. Other symptoms, resulting from experimentally-induced deficiency, have been described, and include:

- impaired growth
- skeletal defects
- loss of reproductive capacity
- prolonged clotting times (not responsive to Vitamin K)
- ataxia
- defective synthesis of mucopolysaccharides in bones and otoliths
- impaired glucose tolerance.

Details of the above symptoms, related to manganese deficiency, are fully described by Lassiter and Hambridge (78).

B.9 **MMT and CMT metabolism, toxicology and elimination**

Methylcyclopentadienyl manganese tricarbonyl (MMT) is extensively converted in vivo to more polar metabolites by oxidation of the methyl group to either an alcohol or an acid. In the rat, about 81% of an orally administered dose of MMT (i.e., 125 mg/rat) is excreted in the urine as one of these two compounds, predominantly (i.e., 83%) as the acid (109). At this high dose, only 2%-4% of the dose is excreted in the feces within the first two days. Earlier work by Moore et al. (135), using a lower dose (i.e., 2.5 mg), showed that 73% was excreted within 24 hours, of which only 36% was excreted in the urine. At an even lower dose (i.e., 0.5 mg), only 68% was excreted.

Until recently, little was known about the distribution of MMT-derived metabolites in the body organs. McGinley et al. (9) injected rats subcutaneously with 4 mg/kg MMT, and showed that blood, lung, kidney and liver manganese concentration levels increased shortly thereafter, the values peaking within 3-6 hours. The lung had particularly high concentration levels at around 3 hours, whereas the liver and kidney had only moderately elevated concentration levels. The accumulation of MMT metabolites preceded maximal pulmonary injury, which occurred within 24-48 hours, suggesting that the accumulation is causally connected to the toxic effects. Brain manganese concentrations were not significantly elevated over control concentration levels. Only 50% of the MMT-derived manganese in the kidney, and less than 2% in the liver and lungs were heptane soluble, indicating that conversion of MMT into a more polar form is rapid in the liver and lungs. Moore et al. (135) have, in fact, shown that liver and lung homogenates rapidly metabolise MMT, whereas kidney and brain homogenates have hardly any activity in this respect.

The data available on the elimination of cyclopentadienyl manganese tricarbonyl (CMT) are not as extensive as for MMT, but there is a suggestion that CMT is converted, by detoxification processes, into an organometallic derivative. Following oral administration of 50 mg/kg to rats, the urinary volume fell rapidly to almost half its original value, while urinary manganese concentration levels increased 50-80 fold over the next two days, during which period about 16% of the administered dose was excreted by this route (136).

Only sketchy details of MMT metabolism are known. Hanzlik et al. (109) confirmed Moore et al.'s earlier findings (135) that mixed-function oxidases (MFO) in the liver and lung catalyze the oxidation of MMT. Hanzlik et al. (109) found that the specific activity of the pulmonary microsomal MFO-catalyzed oxidation of MMT was considerably higher than that for the hepatic microsomal

MFO. Hanzlik et al. (109; 137), as well as Boyd et al. (138), have also shown that phenobarbitol induces increased concentration levels of hepatic, but not pulmonary, MFO; as a result, hepatic clearance of intraperitoneally injected MMT is increased. As a consequence of increased liver clearance, phenobarbitol decreases the pulmonary toxicity of MMT in the rat, even though phenobarbitol does not directly affect pulmonary MFO concentration levels (138). Cytochrome P-450 dependent oxidation of CMT and MMT is known to play a role in detoxification, since pretreatment of rats with piperonyl butoxide (an inhibitor of Cytochrome P-450/MFO system) enhances the toxic effect of these octane boosters (139).

C EXPERIMENTAL STUDIES ON THE EFFECTS OF MANGANESE-CONTAINING COMPOUNDS

The toxicology of both inorganic manganese compounds and of the octane enhancer MMT have been extensively reviewed by Meek and Bogoroch 1978 (3), Bilinski (43), WHO 1981 (15) and, more recently, in a report prepared for the Environmental Health Directorate by MRI in early 1987 (140). For these reasons, section C will emphasize information which has become available during 1985-1987.

C.1 Effects of manganese on specific organs and systems

C.1.1 **Effects on the respiratory system** Over the past two to three years, the main research effort on manganese toxicity has focussed on the CNS, and little new material on the animal toxicology of the respiratory system has become available. A summary of the knowledge to date (taken from references 15; 43; 140) is given below:

Older toxicological studies, summarized in (15), show that female rats, exposed to MnO_2 dust at 0.3 mg/m^3 for 6 months, exhibited peribronchial and perivascular sclerosis, and inflammatory changes. At 1/10th of the dose, no effects were seen (141). Studies by Nishiyama et al. (142) showed that exposure of mice to MnO_2 aerosols (i.e., mmd 1 μm) at concentrations of 0.7 and 3 mg/m^3 for 5 months resulted in inflammatory changes after only two weeks at either doses. The inflammation disappeared after 2 months. Work on guinea pigs, by Rylander, suggests that there may be a synergistic effect on the lungs as a result of MnO_2 accompanying sulphur dioxide. Co-exposure to SO_2 and MnO_2 caused a slower clearance of inert particles, a marked increase in the leukocyte count, and a higher irritation score (143).

In a short-term exposure study, Camner et al. (17) showed that macrophages, washed from rabbit lungs after exposure to an aerosol of $MnCl_2$, were significantly larger than those of controls (14.7 μm vs. 13.3 μm). Exposure was to a 3.9 mg/m^3 aerosol (i.e., mmd of 1 μm) of $MnCl_2$ for 4-6 weeks. At a lower concentration level of $MnCl_2$ (i.e., 1.1 mg/m^3), a slight increase (i.e., 14.2 μm) in macrophage size was observed, but the latter was not statistically significant. Otherwise, no abnormalities were noted. Inhalation of 0.9 mg Mn_3O_4/m^3 aerosols by mice resulted in a 14% reduction in the number of alveolar macrophages, and slightly reduced the phagocytic capability of the lung cells.

In a medium-term study of rats and hamsters, Moore et al. (144) showed that inhalation exposure to Mn_3O_4 at a concentration level of up to 131 μg Mn/m³ for 8 weeks had no effect on the pulmonary system. In other studies (145; 146), rats and squirrel monkeys were exposed to Mn_3O_4 at concentration levels of up to 1152 μg/m³ for 9 months; no evidence of pulmonary dysfunction was seen in the monkeys, although the small size of the groups precludes any definitive conclusions.

C.1.2 **Toxic effects on liver** No recent data are available of the effect of exposure to manganese on the liver function, but older data, taken from a WHO report (15), can be summarized as follows: most of the data concern themselves with massive uptake of manganese (i.e., 10's of mg). In one study on rabbits (147), a daily dose of 3.5 mg $MnCl_2$ was given intravenously for 32 days; the development of hepatic congestion, central vein thrombosis and focal necrosis was observed. In rats, given an intravenous dose of 55-60 mg Mn/kg, there was a decrease in bilirubin clearance from the bile, and a reduction in bile flow. At the higher dose concentration level of 170 mg/kg (148), hepatic necrosis was observed in rats. When rats were given $MnCl_2$ in their drinking water at a concentration level of 200 mg/L for 10 weeks, a histological survey of the liver showed increased amounts of rough endoplasmic reticulum in the centrolobular area, and prominent Golgi in the biliary area, as well as numerous electron-dense mitochondria. Adkins et al. (7) exposed mice to aerosols of Mn_3O_4 of 1.4 μm mmd at 1.8 mg/m³ for 2 hours, and noted liver and spleen oedema throughout the following 2 days (data for the subsequent period are not given).

C.1.3 **Effects on the central nervous system**

C.1.3.1 **Accumulation in the brain following exposure** In section B.4.1, it has already been pointed out that the loading of the mammalian body with manganese results in the temporary accumulation of the metal in most of the internal organs, as well as in the head region and the skeleton. In the rat, Tandon (149) found that manganese accumulated, in order from highest to lowest concentration, in: pancreas > liver > kidney > brain (see also section B.6.3 which outlines the particular susceptibility of the neonate to the toxic effects of manganese).

In mice, the accumulation of manganese in the brain was monitored following a single acute subcutaneous injection of either inorganic manganese, in the form of $MnCl_2$, Mn_3O_4 or organic manganese (i.e., MMT), where all doses were of 0.4 meq/kg (150). The brain was shown to have elevated concentration levels of manganese after 1 day (i.e., 3 to 4 times the control), and these concentration levels were maintained for about one week, particularly when $MnCl_2$ was used, the concentration level being 50% higher than after Mn_3O_4 exposure. The concentration levels subsequently declined rather slowly; the brain concentration levels, at 21 days, were similar to the 1 week concentration levels. No symptoms of toxicity were reported.

Also in mice, Morganti et al. (151) showed that elevated tissue manganese concentration levels resulted from inhalation exposure to an aerosol containing 1.5 μm MnO_2 particles at a concentration level of 49.1 mg Mn/m³ for 12 weeks followed by a 20 week exposure at a concentration level of 85.3 mg Mn/m³. In the cerebrum and cerebellum plus brainstem, the tissue concentration levels, after a

16 week exposure, were 4 and 2.5 times, respectively, those of controls. Thereafter, from about 16-20 weeks, the tissue concentration levels declined slowly (except for those of the liver).

Hietanen 1981 (152) reported that manganese concentration levels in the rat brain peaked at 1 week, following exposure of up to six weeks to $MnCl_2$ in their drinking water. Keen and Lonnerdal (73) showed that intraperitoneal injection of 2.5, 10, or 40 mg Mn/kg in rats rapidly elevated the tissue concentration levels in a dose-dependent manner. Although manganese concentration levels in the brain were not as high as in other tissues (i.e., 0.25-0.75 μg/g), they did not peak or decline as rapidly as in the kidney or liver.

Lopez-Muniz et al. (153) examined the concentration levels of iron and manganese in the rat brain, following inhalation of manganese (unknown form and concentration) for periods of 6 and 9 months. They observed a very pronounced elevation of manganese in the cerebellum, with minor increases in the brain, and intermediate increases in the brain-stem. The amounts in the cerebellum increased three-fold in the period from 6-9 months, as compared to less than 50% in the other tissues.

C.1.3.2 Toxicological effects

Historically, interest in the neurotoxicological effects of acute manganese exposure stems from the observation that certain aspects of the manganism syndrome, exhibited by miners, resemble those seen in Parkinson's disease. It was, therefore, natural that interest should be focussed on the central nervous system (CNS), especially in its catecholamine metabolism and in the morphological changes occurring in the brain, changes which could be responsible for the disturbances in the neurotransmitter systems which exhibit themselves in Parkinson's disease and in acute manganese exposure.

C.1.3.3 Catecholamine metabolism

A number of studies have concerned themselves with the enzymes and intermediates of catecholamine metabolism. The studies have mainly concentrated on the products of tyrosine metabolism, including DOPA, dopamine and epinephrine, as well as with tryptophan and its metabolic products, including tryptamine, 3-indole acetic acid and serotonin. Monoamine oxidase, a mitochondrial enzyme, is of particular physiological importance in the brain, since it inactivates all of the following: dopamine, norepinephrine and serotonin.

Seth and Chandra (154) reviewed the effect of manganese on monoamine concentration levels in the brains of monkeys, rabbits and rats. There are differences between the observations which can be attributed to variations in species, dose, the mode of introduction, and the chemical makeup of the manganese compound. The general conclusion was that long-term chronic exposure to manganese results in a decrease in the concentration levels of dopamine, norepinephrine and serotonin, while short-term exposure either has no effect, or causes a transient increase in amine concentration levels. The significance of these changes in the concentration levels of dopamine and other intermediates is not yet apparent.

Typical illustrations of these temporal changes are provided by Chandra and Shukla (155), and Srisuchart (156). The former have found that manganese treatment initially increased dopamine and norepinephrine concentration levels in the corpus striatum of rats, but that the concentration levels normalized themselves with time, and eventually decreased, such that, by year 1, the concentration levels were significantly lower than in the controls. It is, thus, obvious that the neurotoxic effects of manganese depend on the duration of the manganese insult.

Srisuchart (156) showed that subchronic manganese treatment of mice (i.e., 20 mg/kg intraperitoneally daily for 2 weeks) initially elevated the steady-state concentration levels and turnover rates of dopamine in the corpus striatum. The serotonin and 5-hydroxyindoleacetic acid concentration levels increased in the cerebellum and midbrain, respectively. But, after 4 weeks of exposure, a dose of 10 mg $MnCl_2$/kg reduced the 5-hydroxyindoleacetic acid concentration levels in the cerebral cortex and medulla oblongata, whereas a dose of 1 mg/kg induced the opposite effect in the cortex.

Parenti et al. (157) injected $MnCl_2$ into the substantia negra of rats, which resulted in a dose-dependant loss of dopamine in the striatum, ipsilateral to the injected side. Serotonin and 5-hydroxyindoleacetic acid concentration levels were not affected.

As pointed out by Seth and Chandra (154), very little is known about the effect of manganese on the developing brain. The possible toxicological effects of manganese, as $^{54}MnCl_2$, administered intravenously to pregnant rats, was studied by Kontur and Fechter (158). They showed that, 2 hours after the injection of 4-5 μCi of $^{54}MnCl_2$ only a small percentage of the total dose (i.e., about 0.4%-0.5%) accumulates in the fetus, and, in fact, there was a greater accumulation of ^{54}Mn in placental and maternal tissues. Manganese was administered to pregnant rats as $MnCl_2$ in the drinking water at concentration levels of 0.5, or 10 mg/mL; this resulted in increased concentration levels of manganese in the forebrains of one-day-old rats. The apparent lack of effect of maternally-ingested manganese on the catecholamine metabolism of the fetus contrasts with previously described changes following parenteral administration to mature animals; this demonstrates the efficacy with which the pregnant rat can limit the transfer of manganese to the fetal bloodstream.

Bonilla (159) found that the chronic loading of rats with $MnCl_2$ increased tyrosine hydroxylase activity in rat neostratum 1 month after the start of the treatment, but that enzyme activity was only decreased after 8 months. These results are in accordance with the earlier results of Cotzias et al. (160), who reported an increase in dopamine content in the brain of neonatal mice exposed to manganese. However, Deskin (95) published results which showed that, in neonatal rats exposed from birth to 24 days post-partum, there was a decrease in tyrosine hydroxylase and dopamine concentrations in the hypothalamus. Deskin also looked at the monoamine oxidase concentration levels in the neonate hypothalamus, which, as a result of its good vesiculation, often shows higher concentration levels of manganese than other parts of the brain, and found the enzyme activity had increased by 46% as compared to that in the controls.

C.1.3.4 Receptor binding studies

Seth and Chandra (154) studied the biochemical consequences of long-term manganese exposure in adult rats by measuring the binding of spiroperidol, a dopamine antagonist, to neurotransmitter receptors. This compound binds to striatal cerebellar and frontal cortical membranes, and the authors were able to demonstrate a significant increase in striatal binding in the adult rat following the administration of 10-15 mg manganese/kg. This increase in binding was observed without concomitant increases in the concentration levels of dopamine, 3,4-dihydroxyphenylacetic acid or homovannilic acid. In the cerebellum, binding to GABA receptors was decreased. In the neonatal rat, however, exposure to manganese at day 7 significantly decreased spiroperidol binding to striatal membranes.

Agrawal et al. (123) exposed rats to manganese alone (i.e., 3 mg $MnCl_2$/mL in water), or to manganese and lead (as lead acetate) simultaneously, and showed that coexposure resulted in an increased rate of binding of labelled dopamine to receptors. In equilibrium studies, the binding of tritiated dopamine, norepinephrine or serotonin to corpus striatum membrane fractions was examined; it was concluded that the observed increased receptor binding was, in part, due to changes in the affinity of binding, but that there were also a larger number of binding sites. However, Srisuchart (156) found that specific receptor binding of dopamine, norepinephrine and 5-hydroxytryptamine was unaltered by $MnCl_2$ treatment.

C.1.3.5 **Behavioral studies**

Because of the need to understand the underlying biochemical changes associated with manganism, emphasis has been put on studying metabolism, but some recent papers contain discussions on other aspects of manganese toxicity. Behavioral studies (151) of mice, exposed for 30 weeks to 1.5 μm MnO_2 particles (air concentration levels were not given), showed that, at 16 weeks, small but statistically significant differences in behavioral parameters could be detected (e.g., in exploratory behaviour and rearings in the open field). Interestingly, animals exposed to comparable concentration levels of manganese in the diet did not show any behavioral changes. At no time were any histopathological effects observed.

C.1.3.6 **Other biochemical changes** Anca and Gabor (90) administered chronic doses of $MnCl_2$ to rats through their drinking water (at a concentration level of 10 mg/mL for 1 or 2 months), and showed that blood glucose concentration levels decreased, whereas hexokinase, succinic dehydrogenase, pyruvate dehydrogenase and cholinesterase activities decreased by 20%-80%, in a time-dependent manner in all areas of the brain.

C.1.4 **Other effects**

C.1.4.1 **Effects on other organs** In section B.2.2, it was noted that manganese concentration levels in the testes are, by far, the highest of any tissue (i.e., 4.5 μg/g in Reference Man), suggesting that manganese exposure might lead to toxic effects in this organ, with consequent effects on fertility. In rabbits (161), intravenous administration of $MnCl_2$ at 3.5 mg/kg produced histological changes in the testes, as well as decreases in a number of enzymatic activities affecting germinal activity. Gray and Laskey (162) showed that feeding

manganese to adult mice results in retarded growth and weight of the testes, seminal vesicles and preputial glands.

Buthieau and Autissier (80) treated rats subcutaneously with $MnSO_4$ at a dose level of 10 mg/kg for 5 weeks, and noted significant decreases in serum thyroxine, triiodothyronine, and thyroid stimulating hormone (TSH) concentration levels. They attribute these decreases to alterations, especially of TSH, in the pituitary, in which high concentration levels of manganese accumulated (i.e., 1.76 μg/g in controls vs 63.6 μg/g in exposed animals). However, the concentrations of intrathyroidal hormones were unchanged. Previous work had shown that injection of manganese salts into rats lead to accumulation of manganese in the thyroid (see reference 80 for further references); Buthieau and Autissier confirmed these findings, showing an increase in concentration levels from 6.2 μg/g to 9.2 μg/g. The adrenal glands also showed considerable accumulation of manganese, the concentration level increasing to 8.1 μg/g from 0.9 μg/g in the controls.

Kawada et al. (163) administered $MnCl_2$ to mice at a concentration level of 200 mg/L in drinking water for 7 weeks. As a result, the thyroids of the female mice enlarged 21% as compared to controls, but no goitrogenic effect was observed in male mice unless they were castrated. The goitrogenic effect of manganese in castrated mice could be prevented by testosterone treatment. In agreement with the studies of Buthieau and Autissier in rats (80), serum concentration levels of thyroxine, the main source of which is the thyroid, were reduced, but, in contrast, no change was observed in triiodothyronine concentration levels. Morphological studies showed that the epithelial cells of the manganese-treated female thyroid became flatter, whereas the lumen became enlarged and filled with colloidal material. They speculate that the administration of manganese suppresses pinocytosis and that, as a consequence, colloid is retained in the lumen.

C.1.4.2 **Other toxic effects** Gupta et al. (164) showed that adult monkeys, after an 18-month exposure to high oral doses of $MnCl_2$ (i.e., 25 mg/kg), showed signs of muscular weakness, and rigidity of the lower limbs.

Other effects of exposure to manganese have occasionally been reported in the literature. These include carcinogenicity and mutagenicity (15). A study by Stoner et al. (165) showed that, when manganese sulphate was injected intraperitoneally into mice at a dose of 10 mg/kg once every two weeks over a 30 week period, a statistically significant increase in the incidence of lung tumours occurred. Apart from an earlier study by DiPaolo (166), showing that an 18 month exposure to manganese chloride induced lymphosarcomas in 67% of mice as compared to 24% incidence in controls, there appear to be no other reports of carcinogenicity in the recent literature. In fact, there has been contradictory evidence, including the observation by Furst (167) that rats and mice did not develop excess tumours, and the majority of workers were found to have no increase in manganese, or other trace elements, in cancerous tissues (76). However, Marienfeld and Collins (76) feel that, because of the known mutagenicity of manganese to bacteria and yeast, manganese should not be ruled out as a carcinogen. Mutagenicity of manganese in microbial organisms is better documented, and is reviewed by these authors.

There are a few older studies from the 1960's and 1970's, reviewed in (15), showing that inorganic manganese can induce chromosomal abnormalities in prokaryotes. Baker et al. (168) looked at the frequency of sister chromatid exchange, following exposure of Chinese hamster lung (Don) cells to welding fumes. Fumes from various welding processes were collected, and separated into water-soluble and insoluble fractions before adding to the cell culture. Analysis of the fumes showed various amounts of chromium, nickel, iron and manganese, with the highest manganese content (25%-30%) being found in fume E11, mainly in the form of insoluble oxides. Due to the chemical heterogeneity of the fumes, it is difficult to attribute toxicity to a particular metal compound, but fume E11 had a very low activity in sister chromatid exchange induction, which appears to be mainly associated with the water-soluble chromium component. The soluble fraction of fume E11 was capable of causing mitotic delay, but, of all the fumes, it was the least toxic.

The literature was searched for references to possible allergic responses to manganese, but no evidence was found. Von Rakoski (169) looked at the frequency of reports of allergies against metals used in transplants, and concluded that Cr, Ni and Co could sensitize the body. Patch tests conducted on 370 patients with metal-containing implants showed positive sensitization to chromium, nickel, cobalt and mercury, but not to any of the other metals commonly used in prosthetics (e.g., Be, Cd, Cu, Mo, Zn, or manganese). Caution should be exercised in eliminating manganese compounds from a list of allergens, since it has, for example, been shown that the manganese-containing pesticide, "Maneb", can cause contact dermatitis (170).

Baranes et al. (171) examined the effect of zinc, copper and manganese on the thrombin-induced mast cell degranulation in the mouse, and found that exposure of thrombin to 2-100 μmol $MnCl_2$ prevented degranulation in a dose-dependent manner. Seventy-five per cent inhibition of the granule-associated mediator "ß-hexosaminidase" was achieved at 100 μmol $MnCl_2$, confirming previous findings that manganese can inhibit degranulation in rat peritoneal mast cells. The molecular mechanism for this inhibitory effect is not known, but the results might have bearing on possible inhibition of allergic responses by manganese.

C.2 Toxicological effects of MMT and CMT

The symptoms associated with the administration of MMT and CMT vary in severity, according to the route and dose, but are, generally, similar in the rabbit, rat, mouse, and other species studied. Dermal application, at a dose of 140-795 mg/kg, caused respiratory difficulties, erythema, lung damage, convulsions, oedema, congestion of the kidneys and discolouration of internal organs in rabbits (1). MMT inhalation by rats for up to 4 hours at various concentration levels up to about 300 mg/m^3 resulted in conjunctivitis, dyspnea, and weight loss, but, following the end of exposure, those animals, which had survived, recovered rapidly (1). Short-term inhalation exposure to MMT at a dose of 2 mg MMT/m^3 for 4 hours caused no gross abnormalities in the lungs (Moore et al., quoted in reference 134). Oral administration to rats, or mice, resulted in weakness, diarrhea, discoloration of viscera, necrosis of bronchial epithelia, and diffuse alveolar damage (1; 139; 172; 173). Intraperitoneal injection in the mouse at doses of 100-2000 mg/kg resulted in acute central nervous system (CNS) toxicity, and seizures (174). Oral or intraperitoneal

administration of CMT to rats (136) caused pulmonary oedema, convulsions, lung congestion, and a decline in body weight, with convulsions occurring within 5-10 minutes of administration.

Fishman et al. (174) found that, following exposure of mice to MMT, the concentration of manganese in the brains of mice, showing seizure activity, was higher than in the brains of mice, which did not (i.e., 2.45 μg/g vs. 1.63 μg/g). Moreover, mice treated with $MnCl_2$ showed similar concentration levels of manganese in their brains as those mice with seizures; the absence of seizures suggests that an organic metabolite of MMT, rather than manganese itself, was responsible for the seizures. Evidence was also given, derived from receptor-binding studies, that MMT inhibits the brain GABA-A receptor-linked chloride channel in a manner analogous to that of bicyclophosphorus esters (e.g., picrotoxin), which can also induce seizures.

LD_{50} and LC_{50} values for MMT and CMT have been collected from various sources, and are given in Table C.1. These values compare reasonably well with the data presented by Meek and Bogoroch (3). For example, the mean oral LD_{50} value for rats, from Meek and Bogoroch's Table (n = 9), is 51 mg/kg for males and 38 mg/kg for females.

TABLE C.1 **Toxicity of MMT and CMT**

Compound	Animal	Route	LD_{50}/LC_{50}	Ref.
MMT	Rat	i	76 mg/m³ (4h)	(1)
MMT	Rat	i	247 mg/m³ (1h)	(1)
MMT	Rat	ip	22 mg/kg	(137)
MMT	Rat	o	50 mg/kg (14d)	(137)
MMT	Rat	o	58 mg/kg	(1)
MMT	Rabbit	d	388 mg/kg (1d)	(137)
MMT	Mouse	ip	138 mg/kg (2w)	(173)
MMT	Mouse	ip	152 mg/kg (2h)	(174)
MMT	Mouse	ip	999 mg/kg (2h)	(174)
MMT	Mouse	iv	56 mg/kg	(175)
CMT	Rat	o	22 mg/kg (14d)	(136)
CMT	Rat	ip	14 mg/kg	(136)
CMT	Mouse	iv	3.2 mg/kg	(175)

d dermal ip intraperitoneally i inhalation o oral
iv intravenously h hours d days w weeks

D HUMAN EXPOSURE TO MANGANESE AND MMT

D.1 **Occupational** The main situations, in which the human population can be exposed to hazardous concentrations of manganese, are in industrial environments, typically in the steel industry, and in the mining and processing of manganese ores. As mentioned previously (see section C.2), manganese is not mined in Canada; therefore, the production of manganese alloys in Québec, as well as iron and steel production in Ontario would represent the

main sources of hazard. There are, apparently, no publications dealing with cases of manganese toxicity in a Canadian working environment.

The symptoms of manganese exposure have previously been reviewed in detail (15; 43; 140), and will, therefore, only be summarized briefly. Most of the reported cases of manganese poisoning have involved persons working in manganese mines. The onset, progress and culmination of the toxic symptoms are usually designated as the prodromal and early clinical stages, followed by established chronic manganese poisoning. During the prodromal stage, the symptoms are vague, and include insomnia, headaches, and loss of libido. In the early clinical stage, extrapyramidal disorders set in, with the resultant rigidity of the facial musculature, speech disturbances, and clumsiness of movement. In the final stages, there are reports of rigidity of the limbs and of the face muscles, hand and tongue tremors, muscle pain, asthenia, apathy, and a complex of other psychological manifestations. It was once believed that this syndrome was similar, if not identical, to Parkinsonism, but this view has now been clearly refuted by Barbeau (176).

When the air concentration of fine particles (i.e., 1.0 μm or less) is high (i.e., 1-10 mg/m^3), the incidence of pneumonia is often 2-100 times that of the normal non-exposed population. This type of pneumonia is similar to conventional pneumonia, apart from its resistance to treatment with antibiotics, and the fact that the entire respiratory tract is affected. Clinical signs of chronic manganese intoxication have seldom been reported at exposure concentration levels below 5 mg/m^3. Bilinski (43) states that epidemiological studies in humans indicate effects on the respiratory system at concentration levels below 1 mg/m^3, whereas studies of effects on the CNS, below this concentration level, are inconclusive, or negative.

Details of a dozen epidemiological surveys, made in the 1960's and 1970's, are presented in (15), while further data, extending up to 1985, are to be found in (140). These epidemiological studies have reinforced the clinical picture, as described above, but have not substantially contributed to our understanding of the etiology of manganese toxicity. More recent studies (i.e., 1985-1987), which were not described in the MRI report, are briefly reviewed below.

In section B.2.2, it was noted that the manganese concentration levels in the testes were the highest in the human body; in a subsequent section (i.e., C.1.4.1), studies cited showed that the administration of manganese to rabbits and rats resulted in changes in size, histology, and enzymic activities in the testes. Lauwerys et al. (177) conducted a survey of male workers exposed to mercury vapour, or manganese dust, and found that manganese-exposed fathers had a significantly lower number of children than others in a well-matched control group. The 85 Belgian workers surveyed had been employed in a factory, producing manganese dioxide, carbonate and sulphate from concentrated ores. The air concentrations of manganese were within the range of 0.07-8.61 mg/m^3. The workers were classified into three age groups: 16-25, 26-35 and 36-45 years; it was found that the number of children born to exposed fathers in the first two age groups was half that expected ($p < 0.05$). Diminished libido, or impotence, have frequently been reported in cases of chronic manganese exposure (178; 179).

Jonderko et al. (180) examined the serum immunoglobulin concentration levels in 54 workers, who were exposed for at least five years to high concentration levels of manganese (i.e., 0.2-4.0 mg/m^3) during the manufacture of iron-manganese alloys. Whereas the concentration levels of IgA and IgG, as well as the C_3 and C_4 components of complement, were normal, the IgM concentration levels were depressed by about 21%. In the two control groups, the IgM concentration levels were 102 and 98 mg/100 mL, while, in the exposed groups, the concentration levels were 74 and 84 mg/100 mL.

In a recent paper, Roels et al. (181) examined the effects of manganese exposure on a group of workers in a chemical plant producing various manganese compounds, and, amongst other things, monitored various lung ventilatory parameters, and conducted several psychomotor tests. One of their main findings was that a time-weighted average exposure to manganese dust of about 1 mg/m^3 for less than 20 years can result in preclinical signs of intoxication. Further findings can be summarized as follows:

- Lung ventilatory parameters (i.e., forced vital capacity [FVC], forced expiratory volume [FEV] in one second, and peak flow rate) were only slightly reduced in the manganese-exposed groups, typically around 4%, with only the FVC value being significantly different (i.e., $p=0.05$). There was no interaction between smoking and the spirometric parameters.

- Psychomotor tests revealed that manganese-exposed workers showed a significantly longer mean-reaction-time, performed less well in short-term-memory tests, and showed significantly less precise eye-hand coordination than control workers.

- Statistically different increases in the serum concentration levels of ceruloplasmin (50.9 mg/100 mL vs. 47.1 mg/100 mL in controls), copper (0.107 mg/100 mL vs. 0.101 mg/100 mL), calcium (9.57 mg/100 mL vs. 9.15 mg/100 mL), and ferritin (222 ng/mL vs. 187 ng/mL) were noted.

- White blood cell, and neutrophil, counts were higher in the manganese-exposed groups.

- There was no correlation between simple reaction time, short-term memory, white blood cell, or neutrophil, count, and blood manganese concentration levels. However, correlations were shown to exist between blood-manganese concentration levels and eye-hand coordination, and hand steadiness and serum calcium concentration levels (i.e., $p < 0.02$).

D.2 General population A number of studies on general population exposure have been reported in the various surveys on manganese toxicology (15; 43; 140). In general, it has been difficult to assess these studies, both due to analytical deficiencies, as well as because of the inherent problem of dissociating the effect of manganese from those caused by the other pollutants present in the environment. The general consensus appears to be that long-term exposure to air concentrations at concentration levels generally above 0.2 mg/m^3 can result in an increase in the incidence of respiratory tract ailments.

The Canadian population is normally exposed to low concentration levels of manganese in the environment (see section A.3), with concentration levels in rain, drinking, and lake water being usually less than 20 μg/L (apart from certain regions in Nova Scotia), and air concentration levels of around 0.07 μg/m³ in Ontario. The only situations, in which the general population could be expected to take in amounts of manganese which could be harmful, would be in areas close to industries producing manganese alloys. The WHO has recommended that epidemiological surveys be conducted in areas where the 1-year average manganese concentration is greater than 1 μg/m³. This concentration level has probably not been reached in Canada, although two areas in Ontario have approached this concentration level in 1985 (i.e.,Hamilton-Burlington with a time-averaged geometric mean of 0.408 μg/m³, and Sault Ste. Marie with a corresponding value of 0.378 μg/m³), and this concentration level has even been exceeded, on certain occasions, in Hamilton, Sault Ste. Marie, and Windsor in the same year.

E EVALUATION AND ASSESSMENT

E.1 Evaluation of literature information Since MMT is not produced in Canada, there are fewer occupational risks to persons working within the petroleum industry. In addition, the rapid decomposition of MMT on exposure to sunlight minimizes the health risks associated with the use of the additive. The main concern with the use of MMT is associated with elevated concentration levels of air manganese as a result of MMT's combustion in automobile engines. According to Jaques (4), the combustion of MMT accounted for 17.2% of the total airborne manganese in Canada in 1984 (see section A.2.2.3.1 and Table A.1).

Data on the environmental air concentration levels of manganese in Canada have been difficult to obtain. It has, therefore, been assumed in this report that the Ontario concentration levels are representative of the country as a whole. (see section A.3.1 and Table A.2). The air concentration levels registered in Ontario stations fall within the typical ranges given in the 1981 WHO Manganese report (15) (i.e., 0.01 to 0.07 μg/m³ for urban-rural areas without significant pollution, and up to 0.5 μg/m³ for industrial areas where silicomanganese and ferrous alloys are produced). The maximum air concentration levels recorded in an urban, non-industrialized area (e.g., in Toronto in 1985) were about 0.270 μg/m³, with an annual mean of 0.041 μg/m³. (It is conceivable that air concentration levels of manganese close to Highway 401 in central Toronto would have higher concentration levels, but no data have been found). These concentration levels should be compared with air manganese concentration levels in industrialized areas such as Sault Ste. Marie, where maximum concentration levels of 5.03 μg/m³ were reached, and the time-averaged annual mean was 0.378 μg/m³ (i.e., 10-20 times the Toronto concentration levels). The air concentration levels in industrialized areas of Ontario are approaching the concentration level (i.e., 1.0 μg/m³) at which the WHO recommends that epidemiological surveys be initiated.

The concentration levels of manganese in food and water in the Canadian diet do not give cause for concern, since the uptake of ingested manganese in adults is only about 5.5% of the daily intake, and homoeostatic mechanisms ensure that the daily retention is less than a few micrograms from an intake of 4.7 mg/diem. Animal models suggest that the human body could successfully

eliminate manganese, even if the dietary intake was to be increased ten-fold, or more (i.e., a load of 0.7 mg/kg body weight). An important caveat to such an assessment is that there is a wide individual variation in the ability of the human body to regulate the absorption and elimination of manganese. Thus, Sandstrom (63) noted that absorption was highly variable, ranging from 1.7% to 14.5%, while Mena (116) noted, more than twenty years ago, that there was a large difference in the susceptibility to the toxic effects of manganese amongst exposed miners. However, the concentration levels of manganese intake associated with toxicity are orders of magnitude higher than those to which the Canadian population is normally exposed, such that individual variations in the handling of manganese will not normally be of importance in assessing any possible health hazard associated with the dietary intake of manganese.

The homoeostatic mechanisms of the young mammal are not developed at birth (see section B.6.3), with absorption of manganese approaching 100% of the dietary intake, and excretion, via the bile, being minimal. Under normal conditions, the blood and tissue concentration levels fall to normal concentration levels between the first and second years of life, as the homoeostatic mechanisms mature and the pattern of dietary intake changes. However, up to the age of about 6 months, exposure to heightened loads of manganese, either in the diet, or through lung inhalation, would be expected to increase tissue concentration levels; this would be true especially in the brain, whose blood-brain barrier is poorly developed (see section B.6.3). The risk of increased exposure to manganese, through dietary intake, is minimal, but a possibility exists for increased uptake of airborne manganese particles through the lungs, especially in areas of industrial pollution. Small particles of mmd less than 1.0 μm are of particular concern, since clearance of deposited particulates from the lungs, although initially rapid, is subsequently slow (see section B.4.1, especially B.4.1.2).

The brain and the lungs are the two tissues which have been most thoroughly investigated in toxicological studies (see sections C.1.3.2 and C.1.1), although detrimental effects on other tissues have also been noted (section C.1.4.2). Toxic effects, resulting from exposure to manganese through inhalation, have been well documented in animal studies (section C.1.1), albeit at concentration levels at least 10^3 times higher than the highest concentration levels recorded in Ontario (i.e., an annual geometric mean of 0.4 $\mu g/m^3$ in Sault Ste. Marie). Roels et al. (181) found evidence for preclinical signs of manganese intoxication in humans after exposure to airborne manganese particulates at a concentration level of about 1 mg/m^3 for less than 20 years. Bilinski (43) reached the conclusion that effects on the respiratory system could occur after exposure to air concentration levels of below 1 mg/m^3, and the WHO suggested that epidemiological studies should be carried out when the annual mean concentration in air exceeds 1 $\mu m/m^3$. Considering that the maximum air concentration level of manganese, recorded in Sault Ste. Marie, Ontario, was 5.03 $\mu g/m^3$ in 1985, and placing the minimum toxic air concentration level at 0.2 mg/m^3, it is clear that, even in areas of high industrial pollution, the maximum air concentration levels are still about 40 times lower than the minimum toxic air concentration levels. The contribution of MMT combustion-products to the total air manganese loading in these polluted areas is negligible (see Table E.7).

There may be an effect on male fertility at relatively low air concentration levels of manganese, with exposure of men to air concentration levels ranging from 0.07 to 8.61 mg/m^3 resulting in lowered birth rates (177). However, this concentration level is at least 10^3 times greater than the time-averaged geometric mean value for Ontario (Table A.3).

E.2 Contribution of the combustion by-products of MMT to the total exposure to manganese

In this section, the possible health hazard associated with inhalation of MMT-derived manganese oxides will be assessed from a purely quantitative standpoint. Five Tables are presented (i.e., E.4 to E.8), in which an assessment has been made of the relative importance of manganese uptake from food, water and air. These Tables reveal that young children, and pregnant women, are more exposed to inhalation uptake of toxic particles. For these two groups, uptake of atmospheric manganese through the lungs can contribute about 0.5% of the total manganese uptake from all sources in a worst case scenario (i.e., heavy industrial pollution, with air manganese concentration levels of 0.451 $\mu g/m^3$). [However, since only 17.2% of the manganese in the air can be attributed to MMT combustion, the contribution of MMT-derived manganese oxides to the total manganese uptake will be about 0.1% (or 829 x 10^{-6}, see Table E.7).-not valid]

E.2.1 The intake, uptake and retention of manganese from food and water

E.2.1.1 **Intake** The intake of manganese varies with age. In Table E.1, various estimates of dietary intake, taken from Bilinski et al. (43) and Schlage & Wortberg (108), are given (see also Table B.9).

TABLE E.1 Dietary intake of manganese in the human

Age	Daily manganese intake (mg)	Reference*
3.5 m	0.2	(43)
5.5 m	0.4	(43)
1.5 y	0.8	(108)
4.0 y	1.4	(108)
5.5 y	2.8	(108)
8 y	1.7	(43)
8 y	3.8	(108)
10.5 y	2.0	(43)
11.5 y	2.2	(43; 108)
14.5 y	3.2	(108)
19.0 y	4.2	(108)
25 y**	4.3	(108)

m months y years

* (see Table B.9 for values reported by Schlage and Wortberg (108))

** The relationship between age and dietary intake can be expressed by the equation: $\ln_e y = 0.6504 \ln_e x - 0.5429$ ($r = 0.959$; $p = 0.001$). The daily equation-derived manganese intakes are used in Table E.4 to calculate dietary manganese uptake.

The relationship between age and dietary intake can be described according to the equation:

$$\ln_e y = 0.6504 \ln_e x - 0.5429 \qquad (r=0.959;\ p=0.001)$$

where y is the daily manganese intake, and x is the age in years. This relationship predicts a manganese intake of 2.25 mg for an 8-year-old, 4.71 mg for a 25-year-old and, if extrapolated, 6.40 mg for a 40 year old, values which are well within the ranges quoted in the literature, and in excellent agreement with the value calculated for Canadians (see below and Table A.5).

From the onset of adolescence, males and females have differing dietary intakes. Snyder (47) has assembled data on the variation of the dietary intake of protein, carbohydrate and fats with age. The total food intakes, for a 10-year-old child, an adult female, and an adult male, are 404, 421 and 605 mg/diem, respectively. Thus, the female intake is 421/605 that of the male (or 69.59%), and, using the value of 4.7144 mg Mn/diem (predicted from the previous equation for 25-year-olds), it can be calculated that the dietary intake of manganese for males and females is 5.560 and 3.869 mg/diem, respectively. The dietary manganese intakes for adolescent males and females have been calculated in a similar manner from data given in Snyder (47); a adolescent female's intake is 84.85% of that a male adolescent.

The dietary intake of a pregnant woman is more difficult to assess, since her metabolic rate is elevated. A simple calculation has been used; it was assumed that, as her weight increases by 12.5 kg to about 72.5 kg by full-term, her mid-term weight increase is 10.417%. The manganese intake is, thus, 1.10417 x 3.869 = 4.272 mg/diem.

The estimate of 4.7 +/- 0.8 mg manganese intake/diem, given in Table A.5, was calculated using data from "Apparent per Capita food consumption in Canada" for 1981 and 1982 (182). The manganese contents of the foods were calculated using either the values in Bilinski et al.'s Table 3-25 (43), with preference being given to estimates originating from North America, or using values as tabulated in Geigy's Scientific Tables (42). In many cases, the manganese content of a particular food proved to be quite different, according to the reference values used (e.g., Geigy gives 4.9 mg/100 g for oatmeal, whereas Bilinski et al. gives a value of 0.3 mg/100 g). The total manganese content of the daily Canadian diet, using Bilinski et al.'s values, was 3.727 mg, whereas the Geigy values gave 5.622 mg; a mean value, corresponding to 4.7 mg/diem, has been reported in Table A.5. There are several reasons as to why the dietary concentration levels of foodstuffs, as quoted in the two sources, could be different; on the one hand, the Geigy data are from the 1940's and 1950's, while the Bilinski data, originating from N. America, are from the 1960's and 1970's; improvements in analytical accuracy could account for the generally lower values quoted in Bilinski et al. On the other hand, it is well documented that refinement of foods, in more recent times, has led to a decrease in their manganese content (183).

The dietary intake of manganese from water is, in most cases, minimal; assuming an adult water intake of 1.5 litres/diem, and a manganese content of 20 μg/L (33), only 30 μg Mn/diem will be ingested in fluids; this corresponds to

0.64% of the total dietary intake. Since this figure is far smaller than the s.d., it has not been included in the estimate of total dietary intake.

As noted previously, there is excellent agreement between the dietary intake value, estimated from the double-logarithmic relationship, as compared to the one that was calculated from the manganese content of a typical Canadian diet (i.e., 4.71 mg/diem vs. 4.67 mg/diem).

E.2.1.2 Uptake

The rationale for the uptake figures (i.e., intestinal absorption) is given in section B.1.1, and the data in Table B.1, both of which indicate that, whereas, in the newborn infant, the absorption is close to 99%, the adult absorbs about 5.5% of the ingested manganese. For lack of better data, it is assumed that a 6-month-old child has an intermediate absorption level (i.e., 10%), and that the adult level (i.e., 5.5% absorption) is reached by the age of 1 year.

E.2.1.3 Excretion

Amounts of manganese excreted in the bile are, at best, only informed estimates, with the newborn excreting nothing, and the weanling just over half the dose absorbed from the gut. For adults, a 70% value is close to Mena's 2/3 estimate.

Urinary excretion is usually well below 1% of the intake (see Table B.8), and, in Table E.4, a value of 11% of the uptake (corresponding to 0.605% of intake) has been used for adults, a value close to the one arrived at through balance studies by Spencer et al. (107). Lower values have been chosen for the neonate, and weanling, infant (see Table E.2).

TABLE E.2 **Excretion of manganese expressed as a percentage of the uptake**

Route	Newborn	6-month old	1 year & older
Bile	0	55	70
Urine	1	7	11
Sweat, hair, nails, menses, tears & skin	1	8	15
TOTAL	2	70	96

Other losses (i.e., sweat, hair, nails, etc.) probably account for more of the manganese balance than urinary excretion (see Table E.2 and section B.5.1). The overall balance for the adult human is summarized in Table E.3.

TABLE E.3 Manganese balance in the adult human, oral intake (%)

Intake	Uptake*	Excretion**	Retention***	Mechanism/Route
100.00	-	-	-	Oral intake
	5.50	-	-	Intestinal absorption
		3.85	-	Bile
		0.83	-	Sweat, blood & tears
		0.61	-	Urine
		5.29		Total excreted
			0.21	Retention

* Best estimate arrived at in section E.2.1.2
** Using percentages in Table E.2 for 1 year and older
*** Difference between uptake and excretion

The retention value of 0.21% of intake, arrived at on the basis of the above estimates, corresponds to about 10-11 μg daily, which is a somewhat smaller value than most of the previous estimates (see Table B.8). Matrons et al. (184) estimated a daily retention of about 30 μg Mn/diem on a daily intake of 3 mg manganese (i.e., 1%). However, as discussed previously (in section B.7), the retention value must be small in order to maintain a sensible body burden value within the lifetime of the individual. Data on manganese intake, uptake, excretion and retention are compiled in Table E.4.

E.2.2 Intake, uptake and retention through the lungs

E.2.2.1 Minute volumes and daily air intakes

The minute volumes (M.V.s) were obtained from Snyder et al. (47), with the exception of the one for the 6-month-old infant, the latter being estimated by assuming proportionality between body weight and tidal volume (VT) from birth to 1 year. The daily air intake is equal to (M.V. x 1.44) m^3. Apart from the last set of values (see section E.2.2.4), all calculations are based on resting M.V. values.

E.2.2.2 Air concentration levels of manganese

The three manganese values given are based on the concentration levels predicted in Ontario for 1987 (see Table A.3). Value B is the mean (i.e., 0.0701 $\mu g/m^3$), whereas values A and C represent, respectively, the lower and the upper limits of the range; all are determined by extrapolation of the range limits from 1982 through to 1987 (see Table A.2).

TABLE E.4 Manganese balance in humans (food only), mg/d*

	CHILDREN				ADOLESCENTS		ADULTS		
	6.6d	6m	1y	10y	Female	Male	Female N.P.	Female Preg.	Male
Intake**	.0427	.370	.581	2.598	3.105	3.659	3.869	4.272	5.560
Uptake***	.0427	.037	.032	0.143	0.171	0.201	0.213	0.235	0.306
Excretion									
Bile	.0000	.020	.022	0.100	0.120	0.141	0.149	0.164	0.214
Urine	.0004	.003	.004	0.016	0.019	0.022	0.023	0.026	0.034
Sweat****	.0004	.003	.005	0.021	0.026	0.030	0.032	0.035	0.046
TOTALS	.0008	.026	.031	0.137	0.165	0.193	0.204	0.225	0.294
Non-Absorbed	.0000	.333	.549	2.455	2.934	3.458	3.656	4.037	5.254
Total Excreted	.0008	.359	.580	2.592	3.099	3.651	3.860	4.262	5.548
Retention****** (µg/d)	42	11	1	6	6	8	9	10	12

* All quantities are in mg/d, except for retention, which is in ug/d.

** These daily equation-derived manganese intake values were calculated in section E.2.1.1 above from data in Table E.1 above.

*** Absorption through the gastrointestinal tract is calculated using uptake in Table B.1 multiplied by intake equation calculated in section E.2.1 with data in Table E.1. In the newborn infant, the absorption is close to 99%, whereas the adult absorbs about 5.5% of the ingested manganese. For lack of better data, it is assumed that a 6-month-old child has an intermediate absorption level of 10%, and that the adult level is reached by the age of 1 year.

**** Included in this figure are losses through removal of hair and nails, dermal wear and menstruation.

***** This figure is for the non-absorbed manganese which passes straight through the gut.

****** Retention calculated as intake minus total excreted.

E.2.2.3 **Intake, deposition, absorption and excretion of inhaled manganese** The amount of particulate manganese inhaled into the respiratory tract is calculated as: (daily air intake x air concentration level of manganese). Using the deposition model of Bates et al. (25), it can be estimated that, with particles ranging in size from 0.2 to 2.0 µm in diameter, the total deposition in the respiratory tract will be around 50% of the inspired air concentration. Half of this deposition will probably occur in the lungs. From Table B.2 of this report, it can be seen that, maximally, about 70%

of the manganese oxides deposited in the mammalian lung will be absorbed. Combining these figures, it can be seen that, maximally, 17.5% (i.e., 0.5 x 0.5 x 0.7 x 100) of the inhaled manganese particles will be absorbed onto and through the lung surface. The amount of absorbed manganese that will eventually be excreted is hard to estimate, since experimental data are singularly lacking; the data, reviewed in section B.4.1.2 and Table B.5, indicate that clearance from the lungs is slow. With continuous exposure, through dust inhalation, an overall excretion of only 70% is not unlikely (e.g., 116), giving an overall retention figure of 5.25% (i.e., 0.175 x 0.30 x 100) of the amount inhaled.

E.2.2.4 **Intake of manganese, assuming a heavy work-load**

Upon going from a resting state to heavy work, the minute volume increases by a factor of 5.8108 for men and 5.5556 for women (47) (Note: decimals are given only for calculation accuracy). Assuming a 2:1 partition between resting and hard-work, the resultant factor of 2.6036 for men and 2.5185 for women will permit an estimate to be made for intake, deposition, absorption, and excretion of manganese under conditions of a heavy work-load. However, it should be remembered that deposition factors change considerably with changes in minute volume (i.e., nasal deposition increases, pulmonary deposition decreases while tracheobronchial deposition remains unchanged), such that the values obtained, using these factors, will, at best, only be approximations. All of the respiratory manganese balance figures are given in Table E.5.

E.2.3 **Contribution of uptake of manganese from the lungs in relation to total uptake from all sources**

E.2.3.1 **Lung uptake of total atmospheric manganese** The daily uptake of manganese from the air through the lungs is only a small fraction of the total daily uptake (Note: the latter value is calculated by summing the uptakes from Tables E.4 and E.5). As an example, a 25-year-old pregnant female would be subjected to a total uptake of 235.033 μg for an air concentration level of 0.013 $\mu g/m^3$. Hence, the relative uptake from the lungs is 139 x 10^{-6} (i.e., 0.033/235.033). The values for various ages are presented in Table E.6.

TABLE E.6 Manganese uptake through the lungs as a percentage of the total manganese uptake from all sources*

Air level ($\mu g/m^3$)	CHILDREN				ADOLESCENT			ADULT	
	6.6d	6m	1y	10y	Female N.P.	Male	Female Preg.	Male	
0.013	0.0038	0.0097	0.0143	0.0110	0.0086	0.0085	0.0069	0.0139	0.0079
0.070	0.0207	0.0524	0.0771	0.0592	0.0464	0.0456	0.0373	0.0750	0.0426
0.451	0.1329	0.3368	0.4984	0.3800	0.2982	0.2932	0.2395	0.4813	0.2741

* Fraction of total manganese uptake from all sources that are due to uptake via lungs (10^{-6}), calculated by dividing lung uptake in Table E.5 by the sum of uptakes from diet in Table E.4 and lung above.

TABLE E.5 **Respiratory manganese balance in humans, ug/d**

	CHILDREN				ADOLESCENTS		ADULTS		
	6.6d	6m	1y	10y	Female	Male	Female N.P.	Female Preg.	Male
M.V.*	0.5	1.1	1.4	4.8	4.5	5.2	4.5	10	7.4
Intake**	0.720	1.584	2.016	6.912	6.480	7.488	6.480	14.40	10.66
Mn Intake***									
A.	.009	.021	.026	.090	.084	.097	.084	.187	.139
B.	.050	.111	.141	.484	.454	.524	.454	1.008	.746
C.	.325	.714	.909	3.117	2.922	3.377	2.922	6.494	4.806
Mn Uptake****									
A.	.002	.004	.005	.016	.015	.017	.015	.033	.024
B.	.009	.019	.025	.085	.079	.092	.079	.176	.131
C.	.057	.125	.159	.546	.511	.591	.511	1.137	.841
Mn Retention*****									
A.	.0005	.0011	.0014	.0047	.0044	.0051	.0044	.0098	.0073
B.	.0026	.0058	.0074	.0254	.0238	.0280	.0238	.0534	.0392
C.	.0170	.0375	.0477	.1637	.1534	.1773	.1534	.3412	.2523
Mn Retention Heavy Work******									
A.						.0111		.0190	
B.						.0599		.1021	
C.						.3863		.6569	

* M.V. = lung Minute Volume (L^3/min) from Snyder et al. (47).

** Air intake volume (m^3/d) calculated by multiplying M.V. by 1440 min/d divided by 1000 L^3/m^3.

*** Daily intake via lungs (µg), assuming manganese air levels A,B and C of 0.013, 0.070 and 0.451 µg/m^3, respectively, calculated by multiplying the latter values by the air intake volume; the intermediate level originates from the 1987 Ontario ambient air manganese concentration in Table A.3 above. [approx. 85% unleaded gasoline]

**** Daily uptake via lungs (µg), calculated by multiplying daily intake by the maximum inhaled particulate manganese value from Table B.2 above, multiplied by respiratory tract deposition and by the probability of deposition in the lung from section E.2.2.3 above.

***** Daily retention via lungs (µg), calculated by multiplying daily uptake by the difference (i.e, 30%) remaining after excretion (see section E.2.3.3) derived by Mena et al. (116).

****** Assuming that the person undertakes hard physical work 8 hours daily. All other figures have been calculated in µg, assuming a 24-hour resting state.

E.2.3.2 Uptake of MMT-derived manganese

There are no new data since 1987 on the amount of MMT-combustion attributable manganese in the atmosphere; it is likely that the amount is less than 17% of the total emissions in Canada, since, according to Jaques, the contribution of gasoline combustion to the total concentration levels is steadily decreasing (4). However, a value of 17.2% (i.e., 1984 concentration level in Ontario) has been retained for illustrative purposes. [Note; not valid assumption] Thus, all daily intake, uptake, and retention values in Table E.5 for air concentration levels A and B can be multiplied by 0.172 to give the contribution (in μg) due to MMT combustion. Having done this, the uptake of MMT-derived manganese can be expressed as a fraction of the manganese uptake from all sources; as an example, the non-pregnant adult female exposed to manganese in the air at a concentration level of 0.013 $\mu g/m_3$ (i.e., concentration level A) will have a total uptake from the lungs (see Table E.5) of 0.0147 μg, of which 0.0025 μg is derived from MMT, and 0.0122 μg from other sources. Table E.4 shows that the 25-year-old non-pregnant female will take up 213 μg from food and water, such that the relative contribution of MMT to the total manganese intake from food, water and air is 11.7 ppm (i.e., 0.0025/213.0025).

At air concentration level C (0.451 $\mu g/m^3$), corresponding to areas with significant industrial pollution, it is assumed that the lung uptake of MMT-derived manganese is 21% of the value calculated for air concentration level B in Table E.5 (e.g., 0.079 x 0.21 μg = 0.01659 μg) for an adult female. Since the uptake of manganese through the lungs is small, as compared with the dietary uptake (e.g., 0.511 μg versus 213 μg, respectively for the adult female), the relative contribution of MMT-derived manganese to the total manganese uptake will be approximately the same as that calculated for concentration level B (e.g., 0.01659/213.511 = 78 ppm). The various values are given in Table E.7.

TABLE E.7 Percentage of total uptake from all sources derived from inhaled MMT

Air level ($\mu g/m^3$)	Percentage contribution of MMT-Mn to total uptake*			
	Child(1y)	Female(25y)	Pregnant female	Male(25y)
0.013	25	12	24	14
0.070	133	64	129	73
0.451	857	412	828	471

* MMT-derived fraction of total manganese uptake from all sources (10^{-6}); calculated by multiplying fraction of total manganese uptake from all sources that are due to uptake via lungs from Table E.6 above, by MMT-derived particulate manganese emissions from Table A.1 above.

E.2.3.3 Lung uptake of total atmospheric manganese on a body weight basis

To examine if there was any marked difference in the uptake of manganese through the lungs, when expressed on a body weight basis, the

figures shown in Table E.5 were recalculated as uptake of Mn in ng/kg body weight. Body weights were taken from Geigy (42). As seen from Table E.8, children and pregnant females take up 1.5 to 2 times as much manganese through the lungs as do adolescents and other adults.

TABLE E.8 The uptake of manganese from the air expressed on a body weight basis (ng/kg)*

Air level ($\mu g/m^3$)	CHILDREN				ADOLESCENT		ADULTS		
	6.6d	6m	1y	10y	Female N.P.	Male	Female Preg.	Female	Male
0.013	0.59	0.54	0.51	0.50	0.29	0.31	0.26	0.47	0.34
0.070	2.65	2.57	2.53	2.63	1.53	1.69	1.36	2.50	1.87
0.451	16.8	16.9	16.1	16.9	9.92	10.8	8.81	16.1	12.0
Body** Weight (kg)	3.4	7.4	9.9	32.3	51.5	54.5	58.0	70.5	70.0

* Daily manganese uptake via the lungs on a body weight basis; calculated by dividing manganese uptake via lungs from Table E.5, by body weight.

** Body weights from Geigy (42).

E.2.4 Conclusions to be drawn from quantitative estimates of manganese uptake

The contribution of MMT-derived manganese uptake, via lung inhalation, to the total manganese uptake is normally small, and, for adolescents and adults of both sexes living in rural and urban areas with an average air concentration level of 0.07 $\mu g\ Mn/m^3$, this contribution will be about 0.007% (see Table E.7). However, children and pregnant women, living in these same areas, are more at risk, and the contribution of MMT-derived manganese to the total intake will be about 0.016%. If children and pregnant women are exposed to industrial pollution (e.g., to concentration levels of 450 $\mu g\ Mn/m^3$), air-derived manganese can account for 0.5% of the total manganese uptake from all sources; the relative contribution of MMT-derived manganese will remain small (i.e., approximately 0.008% for an adult female). When the lung uptake of manganese is expressed on a body weight basis (see Table E.8), children and pregnant women take up 1.5 to 2 times as much manganese as do adolescents and other adults.

F CONCLUSIONS AND RECOMMENDATIONS

Neither of the working and general population is judged to be at risk through direct exposure to MMT, since this octane booster is not manufactured in Canada, and is only present at low concentration levels in gasoline. Both the rapid photodecomposition of MMT in sunlight, and its low volatility minimize the potential hazard of this additive.

The oxidation products of MMT, mainly Mn_3O_4, contributed about 17.2% of the air particulate manganese in 1984. The mean annual air concentration level of

manganese in Ontario, for 1987, is estimated to be 0.07 $\mu g/m^3$ (with a range of from 0.013 to 0.451 $\mu g/m^3$), of which MMT-derived manganese oxides contribute half or more for normal cities, and one-sixth or less to industrial cities. The contribution of MMT-derived manganese to the total uptake of manganese from food, water and air is estimated to lie between 0.003% and 0.0162% , while the contribution of total air-derived manganese to total uptake is estimated to lie between 0.004% and 0.5% for ambient concentration levels in 1987.

A potential source of hazard to the general population lies in the inhalation of particulate manganese; but, even in areas of high industrial pollution (i.e., 5 $\mu g/m^3$), the air concentration levels of manganese are at least 40 times lower than the minimum toxic air concentration level (placed at 0.2 mg/m^3). However, children and pregnant women are more at risk than adolescents and other adults, and will take up 1.5 to 2 times as much manganese through their lungs.

Since the incidence of respiratory diseases in infants and small children has been correlated with air pollution, and since the WHO has recommended epidemiological studies when the annual mean air concentration levels of manganese exceeds 1 $\mu g/m^3$ (in two industrial cities, one-half this concentration, on an average basis, was reached in 1987 in Ontario), it is recommended that epidemiological surveys be undertaken in heavily polluted areas (i.e., arterial highways and industrial sites) of Ontario and Québec to assess the degree of correlation between the air concentration levels of manganese and the incidence of pulmonary diseases.

G REFERENCES

1. Hinderer, R.K. Toxicity studies of MMT. Am. Ind. Hyg. Assoc. J., 40 (1979) 164-167.

2. Ter Haar, G.L., Griffing, M.E., Brandt,M., Oberding, D.G. & Kapron, M. MMT as an antiknock: composition and fate of manganese exhaust products. J. Air Pollution Control Assoc., 25 (1975) 858-860.

3. Meek, M.E. & Bogoroch, R. MMT. An assessment of the human health implications of its use as a gasoline additive. Department of National Health and Welfare, Canada. Environmental Health Directorate, Health Protection Branch. 78-EHD-21 (1978).

4. Jaques, A.P. National Inventory of Sources and Emissions of Manganese (1984). Environment Canada EPS 5/MM/1 (1987).

5. Ethyl Corp. Patent No. GB.P. 782738. See Chemical Abstracts 52 3851 (1958).

6. Otto, K. & Sulak, R.J. Effects of manganese deposits from MMT on automotive catalysts in absence and presence of other fuel additives. Environ. Sci. Technol. 12 (1978) 181-184.

7. Adkins, B., Luginbuhl, G.H. & Gardner, D.E. Acute exposure of laboratory mice to manganese oxide. Am. Industr. Hyg. Assoc. J., 41 (1980) 494-500.

8. Garrec, J.-P. & Kudo, A. Repartition et accumulation du MMT dans un écosystème d'eau douce experimental. Environ. Poll. (B) 10 (1985) 173-188.

9. McGinley, P.A., Morris, J.B., Clay, R.J. & Gianutsos, G. Disposition and toxicity of MMT in the rat. Toxicol. Letters 36 (1987) 137-145.

10. Amiard, J.C., Pineau, A., Boiteau, H.L., Metayer, C. & Amiard-Triquet, C. Application of atomic absorption spectrophotometry using Zeeman effect to the determination of eight trace elements (Ag, Cd, Cr, Cu, Mn, Ni, Pb, and Se) in biological materials. Water Res. 21 (1987) 693-697.

11. Ministry of the Environment, Québec. Les methodes d'analyse du cadmium, chrome, cobalt, cuivre, fer, manganèse, nickel, plomb et zinc dans l'eau, les sédiments, les milieux biologiques et l'air. (1981).

12. Zlotkin, S.H. & Buchanan, B.E. Manganese intakes in intravenously fed infants. Biol. Trace Elem. Res. 9 (1986) 271-280.

13. Subramanian, K.S. & Méranger, J.C. Graphite furnace atomic absorption spectrometry with nitric acid deproteinization for determination of manganese in human plasma. Anal.Chem. 57 (1985) 2478-2481.

14. Nagourney, S.J., Heit, M. & Bogen, D.C. Electrothermal atomic-absorption spectrometric analysis of lake waters for Mn, Fe, Pb and Cd. Talanta 34 (1987) 465-472.

15. Elias, P.S. Environmental Health Criteria for Manganese. World Health Organization, Geneva, (1981).

16. Drown, D.B., Oberg, S.G. & Sharma, R.P. Pulmonary clearance of soluble and insoluble forms of manganese. J. Toxicol. Environ. Health 17 (1986) 201-212.

17. Camner, P., Curstedt, T., Jarstrand, C., Johannsson, A., Robertson, B. & Wiernik, A. Rabbit lung after inhalation of manganese chloride; a comparison with the effects of chlorides of nickel, cadmium, cobalt and copper. Environ. Res. 38 (1985) 301-309.

18. Ensor, D.S. & Carr, R.C. Field experience with cascade impactors: quality control of test results. EPA seminar on In-Stack particle sizing for particulate control device evaluations, Meteorology research paper No. MRI 76 PA-1430 (3-4 Dec 1975).

19. Matteson, M.J., Stober, W. & Luther, H. Kinetics of oxidation of sulfur dioxide by aerosols of manganese sulfate. Industrial and Engineering Chemistry Fundamentals. 4 (1969) 677-687.

20. Sanders, J.R. The effect of pH on the total and free ionic concentration of manganese, zinc, and cobalt in soil solutions. J. Soil Sci. 34 (1983) 315-323.

21. Mero, J.L. The mineral resources of the sea. Elsevier, New York (1965).

22. Goldberg, E.D. & Arrhenius, G. Chemistry of Pacific pelagic sediments. Geochim. Cosmochim. Acta 13 (1958) 153-212.

23. Rohbock, E. Atmospheric removal of airborne metals by wet and dry deposition. pp. 159-171. In Deposition of Atmospheric Pollutants, Proceedings, 9-11 Nov 1981. Reidel, Dordrecht, Holland, (1981).

24. Pattenden, N.J., Branson, J.R. & Fisher, E.M.R. pp.173-184. In Deposition of Atmospheric Pollutants, Proceedings, 9-11 Nov 1981. Reidel, Dordrecht, Holland, (1981).

25. Bates, D.V., Fish, B.R., Hatch, T.F., Mercer, T.T. & Morrow, P.E. Deposition and retention models for internal dosimetry of the human respiratory tract. Health Physics 12 (1966) 173-207.

26. National Research Council, Canada. (NRCC #18564). Effects of inhaled particles on human health: influence of particle size and shape. (1982). Based on a report by G.W. Gibbs, M.I. Arhirrii and Y. Doganoglu for the NRCC Associate Committee on Scientific Criteria for Environmental Quality.

27. Chan, W.H., Tang, A.J.S., Chung, D.H.S. & Lusis, M.A. Concentration and deposition of trace metals in Ontario-1982. Water, Air, and Soil Pollution 29 (1986) 373-389.

28. Ontario Ministry of the Environment. Air quality monitoring reports. (1982) Vol I. (ARB 158-84-AQM).

29. Ontario Ministry of the Environment. Air quality monitoring reports. (1983). (ARB-104-85-AQM).

30. Ontario Ministry of the Environment. Annual report air quality in Ontario (1984).

31. Ontario Ministry of the Environment . Annual report air quality (1985).

32. Hem, J.D. Chemical factors that influence the availability of iron and manganese in aqueous systems. Geol. Soc. Am. Bull. 83, p. 443-450 (1972).

33. Department of National Health and Welfare. Guidelines for Canadian drinking water quality - 1978, (1980).

34. Beamish, R.J. & Van Loon, J.C. Precipitation loading of acid and heavy metals to a small acid lake near Sudbury, Ontario. J. Fish. Res. Board Can. 34 (1977) 649-658.

35. Yan, N.D. & Miller, G.E. Characterization of lakes near Sudbury, Ontario. pp.1.1-1.46 In: Studies of lakes and watersheds near Sudbury, Ontario: Final limnological report. Ontario Ministry of the Environment SES 009/82 (1982).

36. Wolt, J. Effects of acidic deposition on the chemical form and bioavailability of soil aluminium and manganese. National Council of the Paper Industry for Air and Stream Improvement, Inc. Technical Bulletin No. 518. (1987).

37. Tabacova, S. Maternal exposure to environmental chemicals. Neurotox. 7 (1986) 421-440.

38. Nakamura, Y. & Osada, M. Studies on trace elements in food and beverages. III. Manganese content in green tea. Ann. Rep. Kyoritsu Coll. Pharm. 3 (1957) 19-21.

39. Brooks, R.R. & Rumsey, M.G. The biogeochemistry of trace element uptake by some New Zealand bivalves. Limnol. Oceanogr. 10 (1965) 521-527.

40. Kirkpatrick, D.C. & Coffin, D.E. The trace metal content of representative Canadian diets in 1970 and 1971. J. Inst. Can. Sci. Technol. Aliment. 7 (1974) 56-58.

41. Méranger, J.C. & Smith, D.C. The heavy metal content of a typical Canadian diet. Can. J. Public Health 63 (1972) 53-57.

42. Documenta Geigy Scientific Tables, 6th Edition, Ed. K. Diem. J.R. Geigy, Basle, Switzerland, (1962).

43. Bilinski, H., Bruins, R.J.F., Erdreich, L., Fugas, M. & Kello, D. Health assessment document for Manganese. U.S.EPA Environmental Criteria and Assessment Office. EPA 600/8-83-013F (1984).

44. Raghib, M.H., Chan, W.Y. & Rennert, O.M. Comparative biological availability of manganese from extrinsically labelled milk diets using suckling rats as a model. Br. J. Nutr. 55 (1986) 49-58.

45. Soman, S.D., Panday, V.K., Joseph, K.T. & Raut, S.R. Daily intake of some major and trace elements. Hlth. Phys. 17 (1969) 35-40.

46. Shiraishi, K., Kawamura, H. & Tanaka, G.I. Daily intake of elements as estimated from analysis of total diet samples in relation to reference Japanese man. J. Radiat. Res. 27 (1986) 121-129.

47. Snyder, W.S. Report of the Task group on Reference Man. International Commission on Radiological Protection, No. 23; Pergamon, Oxford, (1975).

48. Guthrie, B.E. & Robinson, M.F. Daily intakes of manganese, copper, zinc and cadmium by New Zealand women. Br. J. Nutr. 38 (1977) 55-63.

49. Hamilton, E.I. & Minski, M.J. Abundances of the chemical elements in man's diet and possible relations with environmental factors. Sci. Tot. Env. 1 (1973) 375-394.

50. Bohn, H.L., McNeal, B.L. & O'Connor, G.A. Soil Chemistry. Wiley, New York. (1979).

51. Adams, F. & Wear, J.I. Manganese toxicity and soil acidity in relation to crinckle leaf of cotton. Soil Sci. Soc. Am. Proc. 21 (1957) 305-308.

52. Warren, H.V., Delavault, R.E. & Fletcher, K.W. Metal pollution. A growing problem in industrial and urban areas. Can. Inst. Mining Met. Bull. 64 (1971) 34-35.

53. Wright, J.R., Levick, R. & Atkinson, H.J. Trace element distribution in virgin profiles representing four great soil groups. Soil Sci. Soc. Amer. Proc. 19 (1955) 340-344.

54. Shacklette, H.T., Hamilton, J.C., Boerngen, J.G. & Bowles, J.M. Elemental composition of surficial materials in the conterminous United States. U.S. Geological Survey Paper 574-D. Washington D.C. U.S. Government Printing Office (1971).

55. Cotzias, G.C. Manganese in health and disease. Physiol. Rev. 38 (1958) 503-532.

56. McLeod, B.E. & Robinson, M.F. Metabolic balance of manganese in young women. Br. J. Nutr. 27 (1972) 221-227.

57. Kent, N.L. & McCance, R.A. The absorption and excretion of 'minor' elements by man. II. Cobalt, nickel, tin and manganese. Biochem. J. 35 (1941) 877-883.

58. Garcia-Aranda, J.A., Wapnir, R.A. & Lifshitz, F. In vivo intestinal absorption of manganese in the rat. J. Nutr. 113 (1983) 2601-2607.

59. Rehnberg, G.L., Hein, J.F., Carter, S.D. & Laskey, J.W. Age-dependent changes in gastrointestinal transport and retention of particulate manganese oxide in the rat. J. Toxicol. Environ. Health 16 (1985) 887-899.

60. Crounse, R.G., Pories, W.J., Bray, J.T. & Manger, R.L. Geochemistry and man: Health and Disease. 1. Essential Elements. pp.267-333, In Applied Environmental Geochemistry Ed. I. Thornton, Academic Press, London & New York, (1983).

61. Sandstrom, B., Davidsson, L., Cederblad, A., Eriksson, R. & Lonnerdal, B. Manganese absorption and metabolism in man. Acta Pharmacol. et Toxicol. 59 (1986) 60-62.

62. Clarke, R.M. & Hardy, R.N. An analysis of the mechanism of cessation of uptake of macromolecular substances by the intestine of the young rat ('closure'). J. Physiol. 204 (1969) 127-134.

63. Sandstrom, B., Davidsson, L., Cederblad, A. & Lonnerdal, B. A method for studying manganese absorption in humans. Fed. Proc. 46 (1987) 570.

64. Suzuki, Y. Studies on excessive oral intake of manganese. Part 2. Minimum dose for manganese accumulation in mouse organs. Shikoku Acta Med. 30 (1974) 32-45.

65. Van Barneveld, A.A. & Van den Hamer, C.J.A. The influence of calcium and magnesium on manganese transport and utilization in mice. Biol. Trace Element Res. 6 (1984) 489-505.

66. Pollack, S., George, J.N., Reba, R.C., Kaufman, R.M. & Crosby, W.H. The absorption of nonferrous metals in iron deficiency. J. Clin. Invest. 44 (1965) 1470-1473.

67. Greenberg, D.M., Copp, D.H. & Cuthbertson, E.M. Studies in mineral metabolism with the aid of artificial radioactive isotopes. VII. The distribution and excretion, particularly by way of the bile of iron, cobalt and manganese. J. Biol. Chem. 147 (1943) 749-756.

68. Mena, I. The role of manganese in human disease. Ann. Clin. Lab.Sci. 4 (1974) 487-491.

69. Bergstrom, R. Acute pulmonary toxicity of manganese dioxide. Scand. J. Work Environ. & Health. 3 (1977) 7-40.

70. Thomson, A.B.R., Olatunbosun, D. & Valberg, L.S. Interrelation of intestinal transport system for manganese and iron. J. Lab. Clin. Med. 78 (1971) 642-655.

71. Thomson, A.B.R. & Valberg, L.S. Intestinal uptake of iron, cobalt, and manganese in the iron-deficient rat. Am. J. Physiol. 223 (1972) 1327-1329.

72. Flanagan, P.R., Haist, J. & Valberg, L.S. Comparative effects of iron deficiency induced by bleeding and a low-iron diet on the intestinal absorptive interactions of iron, cobalt, manganese, zinc, lead and cadmium. J. Nutr., 110 (1980) 1754-1763.

73. Keen, C.L., Baly, D.L. & Lonnerdal, B. Metabolic effects of high doses of manganese in rats. Biol. Trace Elem. Res. 6 (1984) 309-315.

74. Foradori, C.A. & Dinamarca, M. Transferrin or transmanganin? Definition of the manganese-carrying protein in human plasma. Rev. Med. Chile 100 (1972) 148-153.

75. Suzuki, H. & Wada, O. Role of liver lysosomes in uptake and biliary excretion of manganese in mice. Environ. Res. 26 (1981) 521-528.

76. Marienfeld, C.J. & Collins, M. The ebb and flow of manganese: a possible pathogenic factor in birth defects, cancer and heart disease. Tr. Subst. Environm. Health 15 (1981) 3-20.

77. Papavasiliou, P.S. & Miller, S.T. Generalized seizures alter the cerebral and peripheral metabolism of essential metals in mice. Exp. Neurol. 82 (1983) 223-236.

78. National Research Council, (U.S.), Division of Medical Sciences, Committee on Medical and Biological Effects of Environmental Pollutants. Manganese. National Academy of Sciences, Washington, D.C. (1973).

79. Underwood, E.J. Trace elements in human and animal nutrition. Academic Press, New York, (1971).

80. Buthieau, A.M. & Autissier, N. Effects of manganese ions on thyroid function in rat. Arch. Toxicol. 54 (1983) 243-246.

81. Bencko, V., Geist, T., Arbetova, D., Dharmadikari, D.M. & Svandova, E. Biological monitoring of environmental pollution and human exposure to some trace elements. J. Hyg. Epidem. Microbiol. Immunol. 30 (1986) 1-10.

82. Dupont, C.L. & Tanaka, Y. Blood manganese levels in children with convulsive disorder. Biochem. Med. 33 (1985) 246-255.

83. Roels, H., Lauwerys, R., Genet, P., Sarhan, M.J., De Fays, M., Hanotiau, I. & Buchet, J.P. Relationship between external and internal

parameters of exposure to manganese in workers from a manganese oxide and salt producing plant. Am. J. Industr. Med. 11 (1987a) 297-305.

84. Clegg, M.S., Lonnerdal, B., Hurley, L.S. & Keen, C.L. Analysis of whole blood manganese by flameless atomic absorption spectrophotometry and its use as an indicator of manganese status in animals. Anal. Biochem. 157 (1986) 12-18.

85. Carson, B.L., Ellis, H.V. & McCann, J.L. Toxicology and biological monitoring of metals in humans. Lewis, Chelsea, Michigan, (1986).

86. Mangnall, D., Giddings, A.E.B. & Clark, R.G. Studies of gluconeogenesis in rat liver using a once-through perfusion technique. Effects of manganese ions. Int. J. Biochem. 7 (1976) 293-299.

87. Rognstad, R. Manganese effects on gluconeogenesis. J. Biol. Chem. 256 (1981) 1608-1610.

88. Rorsman, P., Berggren, P.O. & Hellman, B. Manganese accumulation in pancreatic beta cells and its stimulation by glucose. Biochem. J. 202 (1982) 435-444.

89. Vallee, B.L. & Coleman, J.E. Metal coordination and enzyme action, Chapter 6. pp. 165-235 In Comprehensive biochemistry. Eds. Florkin, M. & Stotz, E.H. 12. Elsevier, Amsterdam (1964).

90. Anca, Z. & Gabor, S. Toxic action of manganese on the carbohydrate metabolism in the rat brain. St. Cerc. Biochim. 29 (1986) 24-27.

91. Prasad, K.N. Manganese inhibits adenylate cyclase activity and stimulates phosphodiesterase activity in neuroblastoma cells: its possible implication in manganese poisoning. Exper. Neurol. 45 (1974) 554-557.

92. Keen, C.L., Lonnerdal, B. & Hurley, L.S. Manganese pp.89-132 In Biochemistry of the essential ultratrace elements Ed. Frieden, E. Plenum, New York (1985).

93. Scrutton, M.C., Utter, M.F. & Mildvan, A.S. Pyruvate carboxylase. VI. The presence of tightly bound manganese. J. Biol. Chem. 241 (1966) 3480-3487.

94. Donaldson, J. & Barbeau, A. Manganese neurotoxicity: possible clues to the etiology of human brain disorders. Metal ions in Neurol. Psych. 15 (1985) 259-285.

95. Deskin, R., Bursian, S.J. & Edens, F.W. Neurochemical alterations induced by manganese chloride in neonatal rats. Neurotoxicol. 2 (1980) 65-73.

96. Hirsch-Kolb, H. & Greenberg, D.M. Molecular characterisation of rat liver arginase. J. Biol. Chem. 243 (1968) 6123-6129.

97. Denton, M.D. & Ginsburg, A. Conformational changes in glutamine synthetase from Escherechia coli. I. The binding of Mn^{2+} in relation to some aspects of the enzyme structure and activity. Biochemistry 8 (1969) 1714-1725.

98. Shaw, J.C.L. Trace elements in the fetus and young infant. II. Copper, manganese, selenium and chromium. Am. J. Dis. Child. 134 (1980) 74-81.

99. Mahoney, J.P. & Small, W.J. Studies on manganese. III. The biological half-life of radiomanganese in man and factors which affect this half-life. J. Clin. Invest. 47 (1968) 643-653.

100. Cotzias, G.C., Papavasiliou, P.S. & Miller, S.T. Manganese in melanins. Nature. 201 (1964) 1228-1229.

101. Dastur, D.K., Manghani, D.K. & Raghavendran, K.V. Distribution and fate of ^{54}Mn in the monkey: studies of different parts of the central nervous system and other organs. J. Clin. Invest. 50 (1971) 9-20.

102. Kalliomaki, P.L., Lakomaa, E.L., Aitio, A. & Kalliomaki, K. Manganese distribution in rats after three types of welding fume exposures. Int. Congress Series, Excerpta Med. 676 (1986) 349-352.

103. Morrow, P.E., Gibb, F.R., Lovejoy, F. & McCredie, R. The clearance of radioactive dust from the human and dog lung. Radiation Res. 16 (1962) Abstr. G2 p. 581.

104. Strause, L., Hegenauer, J., Burstein, D. and Saltman, P. The oral assimilation of radiomanganese by the mouse. Biol. Trace Element Res. 7 (1985) 75-81.

105. Stuart, B.O. & Richland, W. Deposition of inhaled aerosols. Arch. Intern. Med. 131 (1973) 60-73.

106. Koldovsky, O., Sunshine, P. & Kretchmer, N. Cellular migration of intestinal epithelia in suckling and weaned rats. Nature 212 (1966) 1389-1390.

107. Spencer, H., Asmussen, C.R., Holtzman, R.B. & Kramer, L. Metabolic balances of cadmium, copper, manganese, and zinc in man. Am. J. Clin. Nutr. 32 (1979) 1867-1875.

108. Schlage, C. & Wortberg, B. Manganese in the diet of healthy preschool and school children. Acta Paed. Scand. 61 (1972) 648-652.

109. Hanzlik, R.P., Bhatia, P., Stitt, R. & Traiger, G.J. Biotransformation and excretion of methylcyclopentadienyl manganese tricarbonyl in the rat. Drug Metab. Dispos. 8 (1980a) 428-433.

110. Mitchell, H.H. & Hamilton, T.S. The dermal excretion under controlled environmental conditions of nitrogen and minerals in human subjects, with

particular reference to calcium and iron. J. Biol. Chem. 178 (1949) 345-361.

111. Weigand, E., Kirchgessner, M & Helbig, U. True absorption and endogenous fecal excretion of manganese in relation to its dietary supply in growing rats. Biol. Trace Element Res. 10 (1986) 265-279.

112. Cirkt, M. Enterohepatic circulation of ^{64}Cu, ^{52}Mn and ^{203}Hg in rats. Arch. Toxicol. 31 (1973) 51-59.

113. Abrams, E., Lassiter, J.W., Miller, W.J., Neathery, M.W., Gentry, R.P. & Scarth, R.D. Absorption as a factor in manganese homeostasis. J. Animal Sci. 42 (1976) 630-636.

114. Maljkovic, T. & Kostial, K. The influence of age on metal toxicity. pp.193-195 In Heavy metals in the environment, 5th Int. Conference, Vol 2, (1985).

115. Davies, N.T. & Nightingale, R. Effects of phytate on intestinal absorption and secretion of zinc, and whole body retention of zinc, copper, iron and manganese in rats. Br. J. Nutr. 34 (1975) 243-258.

116. Mena, I., Horiuchi, K., Burke, K. & Cotzias, G.C. Chronic manganese poisoning. Individual susceptibility and absorption of iron. Neurology 19 (1969) 1000-1006.

117. Chandra, S.V. & Tandon, S.K. Enhanced manganese toxicity in iron-deficient rats. Environ. Physiol. Biochem. 3 (1973) 230-235.

118. Gruden, N. The effect of iron dose on manganese absorption in neonatal and weanling rats. Nutr. Rep. Intern. 34 (1986) 23-27.

119. Kostial, K., Rabar, I., Blanusa, M. & Simonovic, I. The effect of iron additive to milk on cadmium, mercury, and manganese absorption in rats. Environ. Res. 22 (1980) 40-45.

120. Chornock, C., Guerrant, N.B. & Dutcher, R.A. Effect of manganese on calcification in the growing rat. J. Nutr. 23 (1942) 445-458.

121. Chandra, S.V., Ali, M.M., Saxena, D.K. & Murthy, R.C. Behavioral and neurochemical changes in rats simultaneously exposed to manganese and lead. Arch. Toxicol. 49 (1981) 49-56.

122. Eikmann, T., Sels, E. & Einbrodt, H.J. The dependence of manganese excretion in urine on lead concentration in blood. Wissensch. Umwelt 3-4 (1986) 154-155.

123. Agrawal, A.K., Hussain, T., Chandra, S.V. & Seth, P.K. Effects of coexposure of lead and manganese on neurotransmitter uptake and binding in subcellular fractions of rat brain. Biochem. Arch. 2 (1986) 279-285.

124. Schroeder, H.A., Balassa, J.J. & Tipton, I.H. Essential trace elements in man: manganese. A study in homeostasis. J. Chronic Dis. 19 (1966) 545-571.

125. Keen, C.L., Clegg, M.S., Lonnerdal, B. & Hurley, L.S. Whole-blood manganese as an indicator of body manganese. New Engl. J. Med. 308 (1983) 1230.

126. Chan, W.T., Ramadan, T.Z., Perlman, M., McCaffree, M.A. & Rennert, O.M. Copper and manganese in the mother and in the neonate. Nutr. Rep. Int. 22 (1980) 939-948.

127. James, B.E., Hendry, P.G. & MacMahon, R.A. Total parenteral nutrition of premature infants. 2. Requirement for macronutrient elements. Aust. Paediatr. J. 15 (1979) 67-71.

128. Miller, S.T., Cotzias, G.C. & Evert, H.A. Control of tissue manganese; initial absence and sudden emergence of excretion in the neonatal mouse. Am. J. Physiol. 229 (1975) 1080-1084.

129. Kaur, G., Hasan, S.K. & Srivastava, R.C. The distribution of manganese-54 in fetal, young and adult rats. Toxicol. Lett. 5 (1980) 423-426.

130. Shukla, G.S., Dubey, M.P. & Clabaut, J.M. Manganese-induced biochemical changes in growing versus adult rats. Arch. Environm. Contam. Toxicol. 9 (1980) 383-391.

131. Widdowson, E.M. Trace elements in human development. pp.99-117 In Mineral metabolism in pediatrics. Blackwell, Oxford (1969).

132. Casey, C.E. & Robinson, M.F. Copper, manganese, zinc, nickel, cadmium and lead in human fetal tissues. Br. J. Nutr. 39 (1978) 639-646.

133. Doisy, E.A. Micronutrient controls on biosynthesis of clotting proteins and cholesterol. pp. 193-199 In Trace substances in environmental health-VI. Ed. Hemphill, D.D. Columbia, Univ. Missouri (1973).

134. Hurley, L.S., Wooley, D.E., Rosenthal, F. & Timiras, P.S. Influence of manganese on susceptibility of rats to convulsions. Amer. J. Physiol. 204 (1963) 493-496.

135. Moore, W., Hall, L., Crocker, W., Adams, J. & Stara, J.F. Metabolic aspects of methycyclopentadienyl manganese tricarbonyl in rats. Environ. Res. 8 (1974) 171-177.

136. Penney, D.A., Hogberg, K., Traiger, G.J. & Hanzlik, R.P. The acute toxicity of cyclopentadienyl manganese tricarbonyl in the rat. Toxicology 34 (1985) 341-347.

137. Hanzlik, R.P., Stitt, R. & Traiger, G.J. Toxic effects of methyl cyclopentadienyl manganese tricarbonyl (MMT) in rats: role of metabolism. Toxicol. Appl. Pharmacol. 56 (1980b) 353-367.

138. Boyd, M.R., Burka, L.T., Wilson, B.J. & Sasame, H.A. In vitro studies on the metabolic activation of the pulmonary toxin 4-ipomeanol, by rat lung and liver microsomes. J. Pharmacol. Exp. Ther. 207 (1978) 677-686.

139. Haschek, W.M., Hakkinen, P.J., Witschi, H.P., Hanzlik, R.P. & Traiger, J. Nonciliated bronchiolar epithelial (Clara) cell necrosis induced by organometallic carbonyl compounds. Toxicol. Lett. 14 (1982) 85-92.

140. Midwest Research Institute. Health effects of exposure to the gasoline octane booster methylcyclopentadienyl manganese tricarbonyl (MMT) and its major combustion product Mn_3O_4. Contract No. 1508. (1987).

141. Dokucaeva, V.F. & Skvortsova, N.N. Biological effect of experimental inhalation of low manganese concentrations. U.S.S.R. Literature on Air Pollution and Related Occupational Diseases 16 (1966) 98-104.

142. Nishiyama, K., Suzuki, Y., Fujii, N., Yano, H., Miyai, T. & Onishi, K. Effect of long-term exposure to manganese particles. Part 2. Periodical observation of respiratory organs in monkeys and mice. Proc. 45th Ann. Meeting Jap. Soc. Hyg. Med. (Abstr.) Jpn. J. Hyg. 30 (1975) 117.

143. Rylander, R. & Bergstrom, R. Particles and SO_2-synergistic effects for pulmonary damage. pp. A23-A25 In: Proc. 3rd. Int. Clean Air Congress. VDI Verlag Dusseldorf, (1973).

144. Moore, W., Hysell, D., Miller, R., Malanchuk, M., Hinners, R., Yang, Y. & Stara, J.F. Exposure of laboratory animals to atmospheric manganese from automotive emissions. Environ. Res. 9 (1975) 274-284.

145. Ulrich, C.E., Rinehart, W., Busey, W. & Dorato, M.A. Evaluation of the chronic inhalation toxicity of a manganese oxide aerosol. II. Clinical observations, hematology, clinical chemistry and histopathology. Am. Ind. Hyg. Assoc. 40 (1979a) 322-329.

146. Ulrich, C.E., Rinehart, W. & Brandt, M. Evaluation of the chronic inhalation toxicity of a manganese oxide aerosol. III. Pulmonary function, electromyograms, limb tremor, and tissue manganese data. Am. Ind. Hyg. Assoc. 40 (1979b) 349-353.

147. Jonderko, G. & Szczurek, Z. Pathomorphological studies of the internal organs in experimental manganese poisoning. Arch. Gewerbepathol. Gewerbehyg. 23 (1967) 106-116.

148. Baxter, D.J., Smith, W.O. & Klein, G.C. Some effects of acute manganese excess in rats. Proc. Soc. Exp. Biol. Med. 119 (1965) 966-970.

149. Tandon, S.K. Relative distribution of manganese in organs of rat. Ind. Health 11 (1973) 121-125.